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SUCCESSFUL LIGATION OF THE LEFT COMMON ILIAC VEIN FOR THROMBOPHLEBITIS COMPLICATED BY PULMONARY EMBOLI

MAJOR RICHARD T. SHACKELFORD,[1] M.C., A.U.S., AND CAPTAIN RICHARD WHITEHILL, M.C., A.U.S.

Received for publication May 26, 1943

During recent years there has been a great deal of interest and study concerning the treatment of thrombophlebitis. This work has been limited almost entirely to the type of thrombophlebitis which occurs postoperatively, but it applies equally well to cases developing without antecedent operation. The bibliography available to the writers is very limited, and only a few references to the literature can be made. The danger to life of thrombophlebitis arises from the development of pulmonary infarctions. In a large group of patients who had undergone operation, Priestley and Barker (1) found that the incidence of fatal pulmonary embolism was 0.20 per cent. Barker and Priestley (2) in a study of 1,665 consecutive cases of postoperative venous thrombosis, thrombophlebitis and pulmonary embolism, found that about 50 per cent of patients having fatal pulmonary embolism had had previous episodes of non-fatal pulmonary infarction or clinically recognized thrombophlebitis. Of 381 cases of non-fatal pulmonary embolism, 19 per cent subsequently developed fatal pulmonary embolism. To state it another way, and to quote Priestley and Barker, (1) approximately 1 in 4 cases of pulmonary embolism is associated with clinical evidence of thrombophlebitis, and similarly, approximately 1 in 4 cases of thrombophlebitis is associated with evidence of embolism. Of all patients who experience pulmonary emboli, approximately one-half have a single non-fatal embolus, one-quarter have a single fatal embolus, and the one-quarter remaining have multiple emboli, which in approximately 60 per cent of cases are followed, finally, by fatal embolism. Thus, for a patient with a pulmonary infarction caused by an embolus from thrombophlebitis, there is a real danger to his life, and therapeutic measures, if reasonably successful, may justifiably be

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radical. The use of heparin (3) and of dicoumarin (4) can probably decrease somewhat these high mortality figures. Neither substance was available to us and we have had no experience with them. Moreover, we believe that greater success can be achieved by surgical procedures which isolate the site of embolus formation from the general circulation.

CASE REPORT

Private C. G., a 28 year old infantryman, was first seen by one of us (R. W.), January 22, 1943, at the 18th General Hospital. His admission diagnosis was malaria, but his chief complaint was pain in the left upper thigh. His past health had been excellent and his family history was non-contributory, except that a grandmother had tuberculosis when he was a child. On October 13, 1942, he entered active combat in a malaria infested South Pacific island. Malarial prophylaxis consisted of quinine 0.3 gm. daily for one month, and after that, atabrine 48 mg. each day. He first became ill November 3 with pain in the left upper quadrant lasting 4 days. December 9 he developed fever, and this lasted 3 days. He was hospitalized and given quinine and atabrine for what was considered to be malaria. December 15 he again developed fever, and this lasted 3 days. This time he complained of pain in the left lower chest and the pain was increased by breathing. He coughed up dark blood for 5 days, and was told he had pneumonia on the left side. On this occasion he was in the hospital 12 days, and was also treated with quinine and atabrine. He once more developed fever January 7, and thinks it continued for 2 weeks. On January 13 the pain in the chest on breathing returned. This time it involved both sides of the lower chest. The expectoration of dark blood likewise began again, and continued in small amounts until January 18. The fever was not affected by the anti-malarial therapy of atabrine and quinine by mouth, and 5 intramuscular injections of an unknown drug. January 17 he noted for the first time pain along the medial aspect of the left upper thigh. It was not severe and there was no swelling of the leg. Over the next few days it increased steadily. Because of the above sequence of events, he was evacuated by plane to another island January 12, and by boat to this island January 19. As well as he could recall, several blood smears were made, but malarial parasites were not found. On admission to the 18th General Hospital, January 22, 1943, he was complaining of pain in the left upper thigh and in both sides of the lower chest. The latter was aggravated by deep breathing. He was obviously quite ill. He showed evidence of the loss of 50 pounds in weight suffered over the preceding 3 months. T—99.6°F., P—96, R—26, B.P.—110/60. He was poorly nourished. The skin was colored yellow from atabrine. There was no jaundice of the mucous membranes. The head and neck were normal. He coughed occasionally but produced no sputum. Respirations were somewhat rapid and were shallow because of the pain on breathing. The movement of the left side of the chest was restricted more than the right. There was some dullness

and a few rales were heard at the left base posteriorly. The abdomen was flat. The liver and spleen were not felt. The left half of the abdomen was tender, and this was maximal in the lower quadrant. There was tenderness over the medial aspect of the left thigh from 2 inches above the knee to the inguinal region, where there was a little fullness and where the tenderness was greatest. The left thigh measured 16.5 inches and the right 16 inches at a point 10 inches above the knee. He was loath to move the left thigh and held the extremity flexed at the hip and knee. There was no pitting edema of either the thigh or leg. The left ascending lateral branch of the saphenous vein was dilated. There was no other venous distention. Arterial pulsations in the extremities were present and normal. The joints were normal. The neurological and rectal examinations disclosed no abnormalities. Examinations of the urine and blood were not remarkable. The white cell count was 6,100, and a thin blood smear showed no malarial parasites. There was a mild anemia. A portable X-ray of the chest taken the afternoon of admission showed an area of consolidation at the left base in the axilla. The impression on admission was that the patient had a thrombophlebitis involving the left internal saphenous vein, a pulmonary infarct in the left lower lobe, and probably malaria. Treatment with quinine sulfate by mouth was instituted. Over the next two days his condition was unchanged. It was then obvious that if he did have malaria, it was playing no important part in the clinical picture. Surgical consultation was requested with the idea that ligation of the involved vein above the thrombus should be carried out. Accordingly, on January 25 he was seen by the surgeon (R. T. S.) whose impression was thrombophlebitis of the left great saphenous vein, and he recommended ligation of that vein above the site of thrombosis. Sulfathiazole was begun and the drug was continued throughout the active stage of the illness. On January 27, at 8:30 A.M., under sodium pentothal anesthesia, the left great saphenous and femoral veins were exposed below Poupart's ligament. They were found to be completely occluded by a fresh thrombus and the walls were flecked with a fibrinous exudate. Another incision was made above the ligament and the iliac vein inspected extraperitoneally up to the vena cava, and a similar condition was found. The approach was unsatisfactory for further surgical procedure at that time, so nothing was done and both wounds were closed. That evening at 8:00 P.M., the soldier's temperature rose to 103.4°F., slight swelling of the leg became noticeable, and abdominal tenderness became marked. A blood culture taken then was sterile. Abdominal transperitoneal exposure of the pelvic veins was decided upon, and carried out immediately under ethyl chloride induction and ether anesthesia. A low midline incision was made, and the left iliac vein exposed throughout its length, both to the left and the right of the attachment of the mesentery. The entire vein was hard, edematous, and flecked with fibrin up to its junction with the vena cava, which, however, was soft and uninvolved. A tape was placed under the vena cava about 6 cms. above the bifurcation and left loose, for possible later control of hemorrhage. Similarly, a tape was placed beneath the left and right common iliacs 6 cms. distal to their junction with the vena cava. The common iliac was then incised longitudinally between its junction with

the vena cava and the distal tape. There was no bleeding, and a soft clot was seen to fill the entire lumen of the vessel. This was removed with forceps and suction, including a portion which hung loosely in the vena cava. Immediately a gush of blood poured back from the vena cava, requiring the tightening of the tapes for its control. The left common iliac vein was then ligated at its junction with the vena cava with double medium silk, and the two tapes were released. It is possible that every vestige of clot proximal to the ligature was not removed. Another doubled medium silk ligature was placed about the left common iliac vein just below the incision into that vessel, though the clot still was present distal to that level and there was no bleeding from that direction. The raw area was reperitonealized, and the abdomen closed tightly without drainage. At 12 midnight that same evening the temperature rose to 106°F. (rectal), but by 7:00 A.M. the following day had dropped to 100.6°F., and the patient looked remarkably better. His temperature did not exceed that level for 3 days, and he improved rapidly. On Feb. 1, 1943, he developed sudden pain in his right chest, his temperature rose to 101°F., the pulse was 110 and the respirations 34. The physical signs were compatible with those of a small pulmonary infarct, which could have arisen from an incompletely removed clot in the vena cava. He weathered that episode with ease and rapidly improved thereafter, his temperature returning to and remaining normal from Feb. 8 until evacuation to the United States on March 18, 1943. During this time he had no detectable swelling of the left leg while confined to his bed. Four weeks postoperatively he was permitted up and began walking. When ambulant, appreciable but mild swelling of the leg occurred, which was well controlled by the use of an A.C.E. bandage. At the time of his evacuation to the United States on March 18, almost two months postoperatively, he looked well, had regained 25 lbs., and was fully ambulatory. There was minimal swelling of the left leg and this was checked by means of the elastic bandage.

COMMENT

The exact sequence of events in this case is not clear. Whether or not the patient had had malaria, and whether this predisposed to the development of the thrombophlebitis cannot be stated. From the history it is obvious that a pulmonary infarct occurred at least 6 weeks before he was admitted to this hospital. Also from the history it appears that this infarct took place about 5 weeks before the subjective symptoms of the thrombophlebitis. There is nothing to explain the etiology of the thrombophlebitis. There had been no operation and no known trauma. He had been fighting under severe living conditions, had lost a good deal of weight, and probably had had malaria.

We do know that the patient had an extensive thrombophlebitis in-

volving the upper portion of the left femoral and internal saphenous veins, and the whole of the left external and common iliac veins, and that he had had at least 2 episodes of pulmonary infarction. Before operation there was almost no edema of the left leg. This seemed unusual to us, particularly in view of the marked periphlebitis found at operation, and the fact that this would be expected to obliterate the lymphatic channels. In this connection it may be important that there was no evidence of arterial spasm. We were not surprised that edema did not increase after the operation. The veins were already completely obliterated, and ligation and division of the common iliac vein could not increase this obstruction. It could conceivably prevent the subsequent development of recanalization of the veins.

We think that ligation of a vein above the site of thrombophlebitis is the most effective procedure in the prevention of pulmonary infarction. It certainly seems to be a more logical measure than the administration of an anticoagulant. It does not supply the whole answer to the problem, because a large percentage of pulmonary emboli arising from the thrombophlebitis give no signs or symptoms, and are thus not known to exist. The use of venography (5) may reduce this number. Also, the use of anticoagulants postoperatively in patients known to be susceptible to the development of thrombophlebitis, may decrease the frequency of thrombophlebitis.

This experience shows that the common iliac vein can be obstructed and ligated without causing appreciable edema of the leg. How frequently this has been carried out as a therapeutic measure is not known to us. Homans (6) says that if an embolus comes from the external or common iliac vein, it must be regarded, except under the most unusual circumstances, as out of reach.

SUMMARY

1. A case of thrombophlebitis and pulmonary embolism has been presented.

2. Recovery followed ligation of the left common iliac vein at the inferior vena cava.

3. There was very little edema of the left leg before or after the ligation.

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PANTOTHENIC ACID DEFICIENCY IN SWINE[1]

With Particular Reference to the Effects on Growth and on the Alimentary Tract

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Although pantothenic acid is widely distributed in foods, it is possible to produce a severe deficiency in the pig by a very simple means; namely, by feeding a diet consisting of crude casein, sugar, lard, minerals, cod liver oil, and crystalline B vitamins other than pantothenic acid. In fact, on such a diet young pigs develop signs of deficiency much sooner than corresponding pigs receiving calcium pantothenate but lacking any one of the following: thiamine, riboflavin, niacin, pyridoxine, or choline. Inhibition of growth is particularly pronounced and diarrhea accompanying extensive pathological changes in the colon is severe. Sudden collapse occurs and ends fatally. Only less pronounced by comparison with these striking effects, pantothenic acid deficiency in the pig is also characterized by loss of hair, reddening of the skin, excessive nasal secretion, changes in the tongue and degenerative changes in the nervous system associated with the development of marked abnormalities in gait. The last have been described by us already (1). It is the purpose of this report to give an account of the other changes mentioned above.

REVIEW OF LITERATURE

Study of the pathologic changes resulting from a lack of pantothenic acid was first made *in the chick*. In this species a pronounced dermatitis ("chick pellagra") occurs as a result of pantothenic acid de-

[1] These studies were aided by grants from the Rockefeller Foundation, the Fluid Research Fund of the Johns Hopkins Medical School, Parke-Davis & Co., the Upjohn Company, and the Fleischmann Laboratories, and were carried out in coöperation with the Bureau of Animal Industry of the U. S. Department of Agriculture.

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ficiency. This is marked by the formation of a granular and viscous exudate about the margins of the eyelids, crusty scabs at the corners of the mouth, and thickening, cornification, cracking and fissuring of the skin on the bottoms of the feet and between the toes (2). In addition, myelin degeneration of fibers in the spinal cord and chromatolysis of the Nissl substance of nerve cells has been described (3), and it is known that growth is impaired (4).

In the rat not only impairment of body growth and dermatitis but also adrenal hemorrhage and impairment of hair growth with graying or rusting of the hair have been described. Judging by growth response, Unna (5) concluded that a maintenance dose of 80 micrograms of pantothenic acid is required per rat per day. In deficient rats a rough, thin fur, excessive nasal secretion, rusty spots on the fur, sores around the mouth and blood-caked whiskers were described (5). The thinning of the hair became so great that some animals approached complete baldness. The graying of the fur, first noted by Morgan, Cook and Davison (6) is prevented or relieved by the addition of 100 micrograms of calcium pantothenate per day, according to Unna and co-workers (7). Whether pantothenic acid is the only factor concerned in the prevention of nutritional achromotrichia is still disputed, but the observations described above have received adequate confirmation (8, 9). It has been shown that the red deposit around the nose and on the whiskers of pantothenic acid-deficient rats is not blood but coproporphyrin derived from the Harderian (lacrimal) glands (10). The accumulation of coproporphyrin may be due to dehydration (11).

Congestion, fibrosis, scarring and hemosiderin deposition in the adrenals were observed by Daft *et al.* (12) in rats fed a diet deficient in pantothenic acid and some of the animals also showed necrosis, atrophy or hemorrhage as well as fat depletion of the adrenals. Similar observations were reported by others (5, 13, 14) and administration of calcium pantothenate was found to prevent the development of this disorder (13, 14). Control experiments indicated that the adrenal damage was not caused by choline deficiency (13).

In the mouse alopecia, as well as spasmodic muscular movements and eventually paralysis develop in association with pantothenic acid deficiency (15) and myelin degeneration in the sciatic nerves and the

spinal cord has been observed (16). Changes in the adrenals have not been noted.

In dogs Fouts, Helmer and Lepkovsky (17) described intermittent diarrhea which became severe and often bloody before death, decrease in appetite, frequent vomiting, marked loss of weight, great weakness, ulceration of the skin in some animals, and thinning of the hair and slight graying in others, moderate normocytic anemia, decrease in serum proteins and a terminal rise in the blood urea. The administration of glucose and sodium chloride was noted to cause temporary improvement associated with hydremia. At autopsy fatty livers and superficial ulceration of the gums were described. In one dog numerous shallow ulcers and hemorrhages in the ileum were found, and in another a penetrating ulcer on the lesser curvature of the stomach as well as numerous superficial ulcers scattered throughout the stomach and duodenum, were observed. These changes were attributed to "Factor II" deficiency. Relating their observations more specifically to pantothenic acid, Schaefer, McKibbin and Elvehjem (18) ascribed to lack of this vitamin the development of sudden prostration or coma and, usually, rapid respiratory and heart rate, convulsions and severe vomiting. Necropsy revealed fatty livers, "red pigmented spots suggestive of hemorrhagic degeneration" in the thymus glands, dark red kidneys showing macroscopic evidence of "hemorrhagic degeneration" in the cortex and medulla, and frequently gastritis or enteritis and intussusception. The blood glucose and chlorides were lowered irregularly and the non-protein nitrogen values were raised above normal. Acute symptoms were relieved by injection of calcium pantothenate and glucose, but ten to fourteen days were required before appetite improved and weight gain commenced. No macroscopically visible changes were noted in the adrenal glands. No graying of the hair related to pantothenic acid deficiency was observed. "Mottled thymuses" like those described above were observed by Morgan and Simms (19, 20) in dogs and in foxes and they noted graying of the hair in both these species. Scudi and Hamlin (21) reported a lowering of the blood cholesterol, cholesterol ester, lipoid phosphorus, and total lipoids in pantothenic acid-deficient dogs which was restored rapidly to normal by administration of the vitamin. Extremely fatty livers were described in their animals.

The pig was shown by Chick and her co-workers (22), by Hughes (23) and by ourselves (24) to require B vitamins in addition to thiamine, riboflavin, and nicotinic acid. We have shown that the sensory neuron degeneration developing in the nervous system of pigs receiving only these three B vitamins is caused by deficiency of pyridoxine and of pantothenic acid (1). Hughes (25) described in pantothenic acid-deficient pigs decrease in appetite, slow growth, loss of hair, watery and even bloody feces and abnormal gait, with gastritis in all animals and some inflammation in the large intestine and many abscesses in one animal. Similar symptoms have been noted at the Beltsville Research Center (26), where the formation of a brownish-red incrusted material on the eyelids was also noted. In addition to the changes in the nervous system mentioned above, we have reported (1) as resulting from pantothenic acid deficiency very marked impairment of growth, alopecia and reddening of the skin, anorexia, diarrhea, and pathological changes in the colon. These will be described in detail in this report.

MATERIAL AND METHODS

This report will deal chiefly with observations on eighteen pigs. The study of pantothenic acid deficiency was carried out in conjunction with other experiments in which thiamine deficiency (27, 28, 29), pyridoxine deficiency (30), and other forms of nutritional deficiency were produced and at the same time observations were made, for comparison, of pigs fed a complete vitamin mixture or brewers' yeast. "Inanition control" pigs fed a complete diet but only in such quantities that their growth was no better than that of the poorest of the pigs given a deficient diet, were also studied.

Full details of the experimental procedure have been given in the earlier reports already cited. The pigs were received at the laboratory when eleven to twenty-six days of age. All were fed our standard basal diet consisting of Sheffield "New Process" casein, 26.1 per cent; sucrose, 57.7 per cent; lard, 11.0 per cent; and salt mixture No. 3, 5.2 per cent. One "unit" (36.4 grams) furnishing about 152 calories, or a fraction thereof, was given per kilogram body weight per day. To supply vitamins A and D the diet was supplemented with Mead's blended oil, 0.5 gram per kilogram body weight daily. Pigs 7-34 to 7-39, inclusive, were also given Mead's brewers' yeast, one gram per kilogram body weight daily for eleven days. Pigs 7-51 to 7-62, inclusive, received no yeast or B vitamin supplements for three to five days after they were received.

At the ages of thirty-two to thirty-eight days in the case of pigs 7-34 to 7-39, and at sixteen to twenty-two days of age in the case of pigs 7-51 to 7-62, inclusive, the experimental deficiency was commenced. The animals were given the following

crystalline B vitamins: thiamine chloride (0.26 mg. per kg. body weight daily); riboflavin (0.12 mg.); nicotinic acid (1.20 mg.); pyridoxine hydrochloride (0.20 mg.); choline chloride (10.0 mg.); p-aminobenzoic acid (0.10 mg., pigs 7-34 to 7-39; 0.50 mg., pigs 7-51 to 7-62); inositol (0.10 mg., pigs 7-34 to 7-39; 1.20 mg., pigs 7-51 to 7-62).

Observations have also been made on nine pigs, 7-17 to 7-25, inclusive, which received the same vitamin mixture as pigs 7-34 to 7-39 except that no pyridoxine was given.

Under our experimental conditions when all of the above mentioned vitamins are furnished in the quantities indicated, and in addition 0.50 mg. calcium pantothenate is given per kilogram body weight daily, excellent growth takes place and normal development occurs (1). This amount of calcium pantothenate is well in excess of the figure cited by Hughes and Ittner (31) as the minimum requirement for the pig (7.8 to 11.8 mg. daily per 100 pounds live weight, or 173 to 262 micrograms per kilogram). Thus, pigs 7-34 to 7-39 and 7-51 to 7-62, both inclusive, were deficient primarily in pantothenic acid; pigs 7-17 to 7-25, inclusive, were deficient both in pantothenic acid and in pyridoxine.

The crystalline vitamins were given orally by capsule, three times a week.

In order to prevent sudden death of the animals at a very early age, and in order to produce a less acute deficiency than that occurring when no calcium pantothenate is given, certain pigs were fed various quantities of calcium pantothenate as shown in figure 1 and table I.

OBSERVATIONS

The effects of nutritional deficiency in these young pigs became evident in some instances even as soon as eleven days following commencement of the experiment (table I). Diarrhea appeared, the pigs failed to gain weight and lost their appetite, their coats became rough and their hair unkempt so that the thin, "unthrifty" and listless appearance of the pigs contrasted sharply with the well-rounded, lively pigs with smooth coats whose diet differed only in that calcium pantothenate was furnished. The appearance of the pantothenic acid-deficient pigs contrasted even with that of pigs, studied concurrently, whose diets were deficient in thiamine (27) or in pyridoxine (30), for in these more time was required for the effects of the deficiency to become manifest, and even then the visible evidences of deficiency were less pronounced than in those lacking pantothenic acid.

Growth. Comparison of the effects on growth of a lack of thiamine, nicotinic acid, pyridoxine or pantothenic acid (1) indicated that deficiency of the last mentioned vitamin produces by far the most severe impairment of growth in young pigs under the conditions of our ex-

TABLE I

Summary of nutritional and pathologic data in pantothenic acid-deficient pigs

PIG NO.	AGE DEFICIENCY STARTED	AMOUNT CALCIUM PANTOTHENATE GIVEN	AGE AND MANNER OF DEATH*	AVERAGE DAILY WEIGHT GAIN	DIARRHEA		ABNORMAL GAIT—AGE AT ONSET	PATHOLOGICAL FINDINGS		
					AGE AT ONSET	MAXIMUM SEVERITY‡		Colon		Other findings
								Gross	Microscopic	
	days	*micrograms/kg. body wt./day*	*days*	*grams*	*days*		*days*			
7–34	38	50–60 (see fig. 2)	228 D	20	47	+++	93	Hyperemia, wall thickened Abscess, rectum	Chronic ulcerative colitis	
7–35	34	see fig. 2	398 D	27 (to 204 d.)†	45	+++	124	Hyperemia, thickening	Chronic inflammation of wall of colon. Normal mucosa	
7–36	32	see fig. 2	213 K	17 (to 140 d.)†	43	++	107	Normal	Normal	
7–37	32	40	66 D	−19	43	+++	(0)?	Submucosal hemorrhages terminal ileum; diphtheritic colitis	Diphtheritic colitis	Lobular pneumonia
7–38	32	see fig. 2	96 D	60	43	+++	89	Hyperemia	Acute colitis	Lobular pneumonia; fibrinopurulent pleurisy, pericarditis; chronic peritonitis
7–39	32	see fig. 2	230 K	92	44	+++	128	Hyperemia	Chronic ulcerative colitis	Small ulcers, stomach
7–51	22	0	40 K	136	0		0	Normal	Early acute colitis	
7–52	19	0	51 K	129	41	++	44	Hyperemia and follicular abscesses	Marked acute colitis with follicular abscesses	
7–53	20	0	64 D	51	32	+++	43	Hemorrhagic colitis with follicular abscesses	Marked acute colitis with follicular abscesses	
7–54	21	50	40 K	225	0		0	Normal	Slight acute colitis	
7–55	22	50	53 K	190	40	+	0	Hyperemia	Marked acute colitis	
7–56	20	50 (also 1500 × 1)§	78 D	150	39	+++	53	Hemorrhagic and diphtheritic colitis	Severe acute colitis with follicular abscesses	Lobular pneumonia
7–57	21	50	62 D	200	39	++	56	Hemorrhagic and diphtheritic colitis	Severe acute colitis	
7–58	17	50 (also 1500 × 1)	71 D	118	49	+++	58	Acute colitis	Moderate acute colitis	
7–59	16	50 (also 1500 × 3)	82 D	100	51	++	50	Normal	Very slight acute colitis	Lobular pneumonia
7–60	16	50 (also 1500 × 3)	133 K	113	52	++	54	Normal	Slight acute colitis	Abscess, neck; pneumonia
7–61	16	50 (also 1500 × 1)	65 D	86	34	+++	48	Normal	Early acute colitis	Lobular pneumonia
7–62	21	50 (also 1500 × 3)	120 D	133	39	+++	53	Hyperemia	Marked acute colitis	Lobular pneumonia

* D indicates that the animal died, presumably as a result of nutritional deficiency (except 7–38); K means "sacrificed."

† The average daily weight gain for pigs 7–35 and 7–36 is calculated only to the time when treatment with large amounts of calcium pantothenate was commenced.

‡ Severity of diarrhea is represented as follows: +, slight; ++, moderate with mucus; +++, severe, with blood.

§ Pigs 7–56 and 7–58 to 7–62, inclusive, were given single amounts of 1.5 milligrams calcium pantothenate (about 100 micrograms/kg/body wt.) one or three times, at intervals, in order to prevent immediate death from deficiency.

periments. Figure 2 (plate I) illustrates the size of a pantothenic acid-deficient pig as compared with a control animal of the same age. Growth curves of some of the pigs used in this study are shown in figure 1, and their average daily weight gain is given in table I. Pigs in which a "chronic" deficiency was produced by giving small but inadequate amounts of calcium pantothenate (7-34 to 7-39, inclusive) gained no more than 20 to 92 grams daily, and one lost weight. When large amounts of calcium pantothenate were given (7-35, 7-36), weight gain improved considerably (figure 1).

It will be noted in table I that the average daily weight gain in pigs 7-51 to 7-62, inclusive, was greater on the whole than in pigs 7-34 to 7-39, inclusive. In the case of pigs 7-51, 7-52, and 7-53, which were given no calcium pantothenate, this can be accounted for by the fact that two of these pigs were sacrificed at a very early age. The growth of the only animal in this group which died, somewhat later, as the result of deficiency (7-53) was comparable with that of pigs 7-34 to 7-39. Analysis of the growth data for pigs 7-54 to 7-62, inclusive, which were given inadequate amounts of calcium pantothenate, reveals a similar correlation between their average daily weight gain and length of life, longer life tending to be associated with a lower average daily gain in weight.

In contrast to the pigs deficient in pantothenic acid, pig 7-27 (plate I, figure 2), a litter mate of 7-35 which received 500 micrograms calcium pantothenate per kilogram body weight daily and was finally sacrificed at the age of 213 days, gained an average of 433 grams daily. This is representative of the weight gain in animals receiving 500 micrograms of calcium pantothenate in addition to the basal diet and vitamin supplements fed the pigs described in this report.

General appearance, skin and hair. The general appearance of the pantothenic acid-deficient animals has been described above. As the deficiency continued, the hair became thin and tended to fall out, especially over the rump and the center of the back (figure 2). Eventually a patchy alopecia developed, which again was most pronounced over the rump (plate I, figures 3 and 4). In a number of the animals the underlying skin could be seen to be more red than normal and somewhat scaly. Changes in the hair were observed as early as three weeks following commencement of the experiment.

* D indicates that the animal died, presumably as a result of nutritional deficiency (except 7-38); K means "sacrificed."
† The average daily weight gain for pigs 7-35 and 7-36 is calculated only to the time when treatment with large amounts of calcium pantothenate was commenced.
‡ Severity of diarrhea is represented as follows: +, slight; ++, moderate with mucus; +++, severe, with blood.
§ Pigs 7-56 and 7-58 to 7-62, inclusive, were given single amounts of 1.5 milligrams calcium pantothenate (about 100 micrograms/kg/body wt.) one or three times, at intervals, in order to permit immediate tests from deficiency.

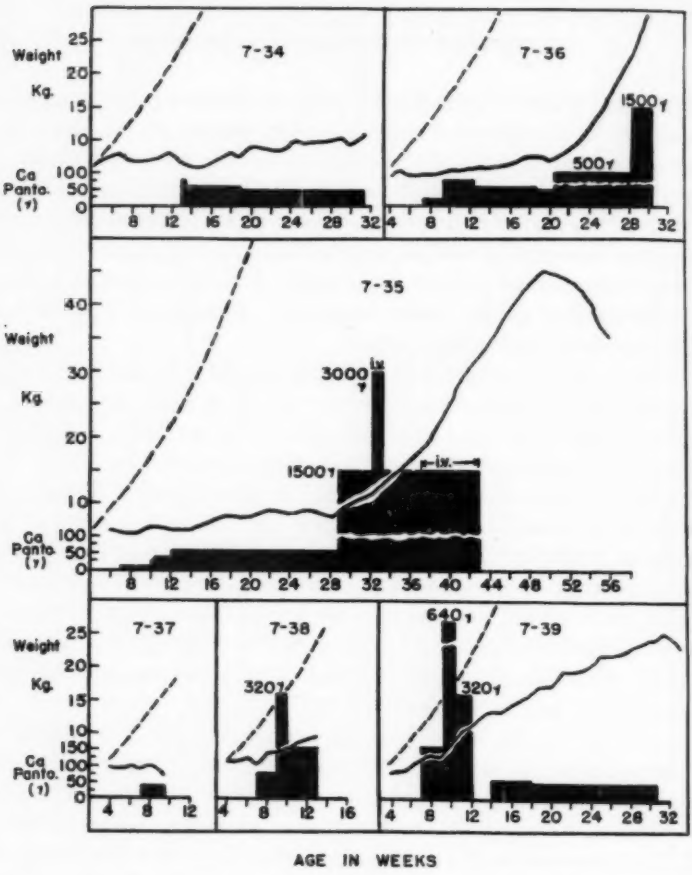

FIG. 1. GROWTH OF PIGS GIVEN VARIOUS AMOUNTS OF CALCIUM PANTOTHENATE, AND RECEIVING AN OTHERWISE SUFFICIENT DIET

The growth of pig 7-38 was probably impaired by an infection which involved the lungs, pleura, and pericardium, and was, no doubt, the cause of death. Pigs 7-34, 7-35, and 7-37 died presumably of pantothenic acid deficiency and were found to have the pathologic changes in the bowel described in this report. Pigs 7-36 and 7-39 were sacrificed. Pig 7-36, which had received large amounts of calcium pantothenate and appeared in good condition except for ataxic gait, showed no pathologic changes at autopsy except in the nervous system. Pig 7-39 showed changes both in the intestines and in the nervous system.

Calcium pantothenate was given orally except when otherwise indicated (i.v. —intravenous).

The broken line, shown for comparison with the pantothenic acid-deficient pigs, represents the growth of pigs given a mixed diet at the Beltsville Research Center and fed and handled according to record of performance procedure.

Alimentary tract. Diarrhea, as already mentioned, was an early symptom in these animals. At first this was not always persistent but after a variable length of time, more or less constant diarrhea developed. The stools were often bright yellow in color and watery. Frequently there was considerable mucus and in many instances a bloody discharge appeared.

Rectosigmoidoscopic examinations were made from time to time in a number of the animals. In two instances these were done twelve days after the deficient diet was started. In pig 7-62 diarrhea had not yet commenced. The rugae were visible and there was edema. There was only slight bleeding at the ano-rectal margin, possibly due to trauma. Diarrhea commenced six days later, and on the following day the second rectosigmoidoscopic examination revealed the mucosa to be confluently hyperemic and slightly edematous. On withdrawing the instrument, bleeding occurred. In pig 7-53 the first examination was made twelve days after the deficient diet had been started, but in this pig diarrhea had just commenced. The bowel was found to be edematous and congested, and liquid feces was present. On swabbing the mucosa, bleeding occurred and withdrawal of the rectosigmoidoscope was followed by profuse bleeding.

As the deficiency continued, in these and in other pigs, the hyperemia of the bowel increased, edema developed, and the rugae disappeared. The tendency of the bowel to bleed after slight trauma became more pronounced. In the fully developed state, the bowel appeared congested and edematous and the slightest trauma, as by gentle swabbing of the bowel, caused bleeding. While the rectum usually showed the most pronounced changes, it could be seen that the bowel was affected for the full 25 centimeters which could be observed through the rectosigmoidoscope; that is, the rectum and the lower sigmoid. The contents of the bowel often contained no stool or a small quantity mixed with blood and occasionally muco-pus. At no time, however, could ulceration of the mucosa be seen.

Attempts were made to examine the bowel by barium enema, but no satisfactory films were made.

Patchy areas of reddening at the margin of the *tongue* or on the lip, often surrounded by a serrated border of white, were noted from time to time. These changes were irregular in their development and

became more or less pronounced from time to time. In a few instances, shallow ulcerations developed on the sides of the tongue, and in other pigs there appeared to be some papillary atrophy. In some of the pigs such changes were observed as soon as three weeks following institution of the experimental deficiency, but in about a third of the animals abnormalities in the tongue were never noted.

Gastric analysis, following the injection of histamine phosphate, (10 micrograms per kilogram body weight) was carried out in six pigs when they were over six months of age, at a stage of severe deficiency. No free hydrochloric acid was found in one animal and values ranging from 10 to 18 units for free hydrochloric acid and 15 to 56 units for combined acid were found in the remainder. In our experience, these findings are not abnormal.

Respiratory tract. When the deficiency became pronounced, cough developed in many of the pigs and was accompanied by a watery discharge which often could be seen dripping from the snout.

Nervous System. The time of onset of abnormality in the gait of the animals was definitely related to the time at which the experimental deficiency was commenced. In pigs 7-51 to 7-62, inclusive, which had been deprived of pantothenic acid since the age of sixteen to twenty-two days, a slightly stiff-legged gait with a slight tendency to cross the hind legs ("scissors gait") could be detected after about five weeks, and in two instances (7-52 and 7-53) were seen even sooner. Well defined ataxia was evident after another week or two. In pigs 7-34 to 7-39, which had received yeast for eleven days after they arrived at the laboratory and in which the experimental deficiency was not commenced until they were thirty-two to thirty-eight days of age, slight abnormality in gait was not observed before eight to thirteen weeks. These pigs were given various amounts of calcium pantothenate (figure 2) with the object of producing a "chronic" deficiency, and well-defined ataxia did not appear until the animals were thirteen to twenty-three weeks of age. The changes in the nervous system associated with pantothenic acid deficiency have been discussed elsewhere (1, 32).

Blood—Morphology. No severe anemia, such as that which occurs in pyridoxine deficiency (30), developed in the pantothenic acid-deficient pigs. A moderate, normocytic anemia was noted in thirteen out of

eighteen pigs. In seven the volume of packed red cells ranged between 35 and 40 cubic centimeters per 100 cubic centimeters of blood. In the remainder, the volume of packed red cells ranged between 24 and 33 cubic centimeters. In pigs receiving an adequate diet we have found the volume of packed red cells to range from 41.5 to 51.5 cubic centimeters (33).

In the two animals receiving treatment with calcium pantothenate (7-35 and 7-36) the anemia became less pronounced, the volume of packed red cells rising from 25 to 38 cubic centimeters and from 33 to 39 cubic centimeters per 100 cubic centimeters of blood in the two pigs, respectively. Changes in hemoglobin and red cell count were, in general, parallel with those in volume of packed red cells as no significant alteration in the mean size or hemoglobin content of the red corpuscles took place.

Chemistry. In view of the sudden collapse observed in pantothenic acid deficiency, as well as because pathologic changes have been described in the adrenals of pantothenic acid-deficient rats (12, 13, 14), chemical studies of the blood of pigs in various stages of pantothenic acid deficiency were made. A summary of some of these data is presented in table II. A consistent occurrence was a fall in the plasma chlorides. Less regularly, the CO_2 combining power of the blood tended to rise. Only in an extreme stage of deficiency, as in pig 7-34, was the nonprotein nitrogen of the blood raised substantially. The severe hypoglycemia which was observed in dogs by Schaefer *et al.* (18) was not a feature in the pigs. No consistent alteration in the plasma protein content took place in our animals. It is noteworthy that the administration of 60 micrograms of calcium pantothenate per kilogram body weight to pig 7-34, an amount sufficient to bring about some improvement, but by no means enough to cause cessation of diarrhea or much gain in weight (figure 1), was associated with a marked decrease in nonprotein nitrogen, a fall in blood CO_2, and a rise in plasma chlorides. The plasma chlorides, however, did not reach normal levels on this dose. In pig 7-35, the administration of 1500 micrograms per kilogram body weight, an amount which brought about great improvement and gain in weight, was associated with a restoration of plasma chlorides to normal, a marked rise in blood sugar, and a fall in blood CO_2.

TABLE II

Chemical findings in the blood of normal and of pantothenic acid-deficient pigs, as well as in pigs with other types of vitamin deficiency

TYPE OF ANIMAL	AGE	CALCIUM PANTOTHENATE GIVEN		CONDITION	FOOD CONSUMPTION	BLOOD						URINE	
						Serum					Non protein nitrogen	Glucose	
		Amount	Duration			CO_2	Cl_2	Na	K	Total protein			Cl / Na
	days	*γ/kg./day*	*days*		*units**	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*grams per cent*	*mg. per cent*	*mg. per cent*	*m.eq./24 hr.* / *m.eq./24 hr.*
Normal, average (5 pigs)	54–241	500	20–220	Excellent	1	25.7	107.1			6.12	26	57	
Thiamine-deficient	107	500	69	Good	1	21.0	101.4			6.05	43	75	
Pyridoxine-defic., average (11 determinations in 4 pigs)	118	500	84	Good	1	20.0	101.0			6.27	37	73	
Panto-deficient treated	72	160–640	21	Good	1	28.2	100.8			4.74	32	69	
Panto-deficient average (6 pigs†)	39–51	0	19	Fair, slight diarrhea	1	24.2	102.1			5.77	38	69	
Panto-deficient average (5 pigs‡)	56–62	0	24	Diarrhea	¼	24.6	99.0			5.42	30	58	
Panto-deficient average (3 pigs§)	57–61	0	25	Diarrhea	¼	25.5	99.3			5.54	36	65	
Panto-deficient (pig 7-55)	52	0	30	Diarrhea	1	32.3	96.8			6.63	36	84	82.6 / 77.4
Panto-deficient (pig 7-52)	51	0	32	Diarrhea	1	34.8	90.6	144		5.63	38	75	46.8 / 28.5
Panto-deficient pig 7-34	58	0	20	Weak, diarrhea	¼	19.8	95.0			5.40	36	70	
7-34	71	0	33	Thin, diarrhea	1	18.5	89.8			6.79	58	75	
7-34	76	0	38	No weight gain, diarrhea	1	25.2	88.8			5.83	24	81	
7-34	89	0	51	Has lost weight, diarrhea	0	26.0	79.6	120	3.7	5.90	116	66	1.9 / 00.0
7-34	91	80 im.	1		0	27.3	77.4			5.66	84	88	
		60	2										
7-34	192	60	103	Improved, but weak, diarrhea	¼	20.6	90.0			6.96	36	39	
Panto-deficient pig 7-35	54	0	20	Thin, diarrhea	¼	19.4	92.8			6.64	28	45	
7-35	74	10	12	Thin, diarrhea	1	23.1	96.0			8.19	30	43	
		40	8										
7-35	178	50–60	104	Thin, slight weight gain	¼	10.0	97.8	135	5.8	8.02	26	56	
7-35	203	50	25	Condition poor	¼	24.4	82.6			8.12	27	64	
7-35	245	1500	42	Much improved	1	18.1	102.4			6.28	30	139	

* Each unit represents 36.4 grams of the basal diet per kilogram body weight

† Pigs 7-36 to 7-39, inclusive, 7-51 and 7-54

‡ Pigs 7-17 to 7-20, inclusive, and 7-24

§ Pigs 7-22, 7-23, and 7-25

Pathological anatomy. Pantothenic acid deficiency produces the most striking degree of malnutrition that we have encountered in studying various types of "B vitamin" deficiencies in the pig. The skin, dirty, wrinkled, somewhat scaly and largely devoid of hair, as already described, was supported by almost no subcutaneous tissue. The genital glands seemed infantile in appearance. In a few of the animals, infections about the hoofs and in the lungs (pneumonia) were present. The most pronounced anatomical changes observable on gross examination were found in the gastro-intestinal tract. These were present in all of the animals dying of pantothenic acid deficiency (table I).

Grossly, the earliest change in the *colon* consisted of a diffuse hyperemia. This was found after four weeks. An exudate, which appeared as flecks in early cases, became confluent in the more advanced stages of deficiency. The lymphoid follicles were found to be increased in size and on cross section contained purulent centers. Perforation of these abscesses produced small ulcers. Larger ulcers covered with grayish-green exudate were also found and the wall of the colon beneath was indurated. The serosa in the more advanced cases was more opaque than normal, and numerous fibrous adhesions were found. The mesenteric lymph nodes from the very beginning were enlarged, and on section the soft lymphoid tissue bulged above the capsule.

Microscopically, changes could be seen in the colon as early as the eighteenth day. The normal colon is lined by glandular mucosa made up of cells containing large vacuoles (plate II, figure 6). There are many scattered nodules of lymphoid tissue in the submucosa. The initial change to be observed seemed indicative of injury to the cells lining the glands. The mucus vacuoles disappeared and the cells became atrophic. In addition, leukocytes, polymorphonuclear as well as mononuclear, appeared in the lumina of such glands and in the periglandular interstitial tissue (plate II, figure 7). This change was not a diffuse one in the early stages of the deficiency as only one or two glands here and there were involved (plate II, figure 6). However, more and more glands showed this change in the more advanced instances of deficiency so that the process in severe cases was a diffuse one. In such animals the lumens of the glands were filled and overdistended with polymorphonuclear leukocytes while the lining epi-

† Figs 7-36 to 7-39, inclusive, 7-51 and 7-54
‡ Figs 7-17 to 7-20, inclusive, and 7-24
§ Figs 7-22, 7-23, and 7-25

thelium was atrophic and flattened. In most instances the superficial glandular cells were necrotic (plate II, figure 8) and a diphtheritic exudate appeared on the surface. Coincident with these changes, lesions were found in the lymphoid tissue of the submucosa. Normally, prolongations of glandular elements are found in the solitary lymphoid follicles (plate III, figure 9). Changes were present in these glands like those seen in the mucosal glands. The lumens were distended by accumulations of leukocytes and the surrounding lymphoid tissue was hyperplastic and infiltrated with polymorphonuclear cells. It was evident that the mucosal cells had died and there resulted an abscess in the center of a greatly enlarged lymphoid follicle (plate III, figure 10). With necrosis of the overlying tissue, ulcers developed. These ulcers tended to be larger in size in the more severely deficient pigs. Necrosis of the superficial mucosal glands also contributed to the enlargement of the ulcers.

In the *adrenal glands* no alterations were found microscopically. The histology of the *liver* was not remarkable. In the *skin*, the most conspicuous change microscopically was atrophy of the epidermis. This layer was reduced in thickness and the cells were decreased in size. In the more severe cases, there were small superficial ulcers. The hair follicles also showed atrophy and in the severe cases had disappeared.

Sections of the *tongue* showed a non-specific subacute inflammatory reaction. In several instances there were ulcers whose bases were made up of granulation tissue covered with desquamated cells and bacteria. Leukocytes were found in the underlying muscle and connective tissue. In places, the epithelium was hyperplastic, but this was always associated with areas of inflammation.

EFFECTS OF ADMINISTRATION OF CALCIUM PANTOTHENATE

A number of the pigs were given various amounts of calcium pantothenate, with the object of preventing early death and in order to produce a chronic deficiency. It is evident that amounts up to 60 micrograms per kilogram body weight daily, given by mouth, were substantially below the requirement (figure 1, and table I) and failed to prevent the development of the picture of pantothenic acid deficiency. Pigs receiving 80 or 160 micrograms (figure 1, pigs 7-38 and 7-39) fared better: their appearance was cleaner and less gaunt, the hair

was in somewhat better condition and diarrhea was absent or not severe. Control animals receiving 500 micrograms per kilogram body weight daily have always grown satisfactorily and none of the effects ascribable to pantothenic acid deficiency have been observed. This amount given therapeutically (figure 1, pig 7-36) produced a gain in weight, cessation of dysentery, improvement in the condition of the skin and growth of hair as well as moderate improvement in gait. Still greater amounts of calcium pantothenate (figure 1, pig 7-35), however, failed to bring about a complete return of normal function of the nervous system once well-pronounced ataxia had developed.

In pig 7-35 repeated rectosigmoidoscopic examinations were made and the effects of treatment noted. Dysentery ceased within two days after the administration of 1500 micrograms calcium pantothenate per kilogram body weight daily. After three weeks, however, the inflammatory changes in the lowermost 12 centimeters of the bowel could still be observed, although above this point a nearly normal appearance had returned. After nine weeks of treatment there was considerable improvement. Instrumentation no longer caused bleeding, although swabbing still produced slight hemorrhage. The rugae could be seen, there was practically no edema and the bowel was of good color. Treatment was continued for another six weeks and the condition of the bowel remained about the same. Cessation of treatment did not lead to a recurrence of diarrhea or to abnormal appearance of the bowel until twelve weeks later when diarrhea was present, the bowel was once more hyperemic and edematous, and bleeding occurred readily on swabbing. It is noteworthy that this animal continued to gain weight for eight weeks after no more calcium pantothenate was given.

In the intestines of animals which had been treated, it could be seen at autopsy that ulcers had healed, but the wall of the colon was thicker than normal. Microscopically, the epithelium was found to be regenerated and the glands were more normal in appearance. Nevertheless, there remained a good deal of round cell infiltration in the mucosal interstitial tissue, in the submucosa, and in the muscular layers. An increased amount of connective tissue was also found in the wall of the large intestine (plate IV, figure 12).

The histology of the changes in the nervous system and the effects of treatment thereon will be discussed elsewhere (34).

DISCUSSION

The outstanding effects of pantothenic acid deficiency in the pig as indicated by these studies are impairment of growth, diarrhea, and even dysentery with colitis, impairment of appetite, loss of hair and the development of abnormality in the gait which is associated with histologic changes in the nervous system. The skin becomes red and scaly, lesions on the tongue may appear and excessive nasal secretion together with cough often develop.

The impairment of growth which occurs in pigs deficient in this vitamin is more pronounced than we have observed in association with deficiency of thiamine or pyridoxine. However, how much of this impairment of growth is attributable to the diarrhea and consequent failure of absorption, as well as to the loss of appetite which soon develop in pantothenic acid-deficient animals, is difficult to state.

Diarrhea is frequently observed in association with nutritional deficiency, both in man and in experimental animals. Improvement in pellagra following the administration of nicotinic acid is accompanied by cessation of diarrhea. In swine a syndrome has been observed (35, 36, 37) which is manifested by loss of appetite, anemia, dermatitis and diarrhea. At autopsy in many instances necrotic enteritis was found and the entire syndrome has been called "pig pellagra." In the treatment of this condition, nicotinic acid seemed to be of value. It is likely, however, that other essential foodstuffs were also lacking, such as proteins and vitamins of the B complex. The role of nicotinic acid in "pig pellagra," in our opinion, needs reëvaluation. In our own experiments with B vitamin deficiencies in swine, diarrhea has not been present in pigs given no nicotinic acid (38). It has been sometimes seen in pigs lacking pyridoxine or riboflavin, but has occurred consistently and continuously only in those lacking pantothenic acid. The pathologic changes in the bowel which are here described have been seen only in those animals which were deficient in pantothenic acid. Powick (39), on the other hand, in experiments being conducted at the Bureau of Animal Industry of the U. S. Department of Agriculture, has found diarrhea a prominent symptom in young pigs fed a synthetic ration devoid of nicotinic acid.

It is interesting to speculate concerning the pathogenesis of the diarrhea and the cause of the changes in the colon. Morphologically

the initial change in our pantothenic acid-deficient pigs was in the mucosal cells. There was a loss of secretory vacuoles, followed by atrophy. In the more advanced instances leukocytes were present in the mucosa and submucosa and the lining cells showed further change and necrosis. The possibility, therefore, arises that the changes in the bowel may be due to the absence of mucous secretion or perhaps are caused by the lack of some enzyme or enzymes normally secreted by the colonic epithelium. Whether this could produce the changes is hard to say. One must also consider the possibility that there is a change in the bacterial flora in the bowel and an increase in pathogenic organisms with consequent damage to the lining epithelium and irritation of the muscularis. Both of these factors may be important and one may complement the other.

The appearance of the bowel of these animals has impressed us particularly because of its similarity on proctoscopic examination to that of chronic nonspecific ulcerative colitis as seen in man. The etiology of this disorder is unknown and one wonders whether nonspecific ulcerative colitis, as well as certain instances of diarrhea in children, may not be caused by a lack of pantothenic acid. With the present evidence, however, this is a matter only for speculation.

It is not necessary to postulate alterations in secretion of adrenal cortical hormone (40) in order to explain the chemical changes which have been found in the blood of the pigs. In contrast to the sodium loss seen in Addison's disease, pig 7-34 showed a capacity to conserve sodium when the blood level had fallen, for at that time none was excreted in the urine. The drop in serum chlorides which accompanies pantothenic acid deficiency is more likely due to inanition and diarrhea; the rise in the carbon dioxide combining power of the blood is probably a compensating change. The sharp rise in nonprotein nitrogen in the terminal stage of deficiency may possibly be due to renal shutdown. We have observed no consistent alterations in glucose, and it is to be noted that the findings of Schaefer *et al.* (18) were quite irregular. Consistent with these conclusions is our failure to make out pathologic changes in the adrenal glands.

SUMMARY

Pantothenic acid deficiency in the young pig was characterized by diarrhea, then dysentery, and loss of appetite, very marked impair-

ment of growth, loss of hair, cough and excessive nasal secretion, changes in the tongue, and abnormal gait.

There was extensive colitis characterized by hyperemia, edema, and ready bleeding of the bowel when examined rectosigmoidoscopically. At autopsy the changes ranged from diffuse hyperemia, increase in the size of the lymphoid follicles and the formation of small ulcers, to extensive inflammatory changes involving the large intestine. Histologically, atrophy of the cells lining the glands of the mucosa, abscess formation, and ulceration were observed.

Deficiency was accompanied by the development of a moderate normocytic anemia, a fall in serum chlorides, increase in the carbon dioxide combining power of the blood, a terminal rise in nonprotein nitrogen and sometimes hypoglycemia.

The administration of 500 or more micrograms calcium pantothenate per kilogram body weight per day was accompanied by cessation of diarrhea, gradual improvement in the condition of the bowel, a restoration of normal blood values, growth of hair, and gain in weight.

The abnormal gait, which was associated with well-defined histologic changes in the nervous system, improved following administration of calcium pantothenate, but complete restoration of function did not occur.

Vitamins used in these experiments were provided by Merck & Co., Inc. and yeast and cod liver oil by Mead, Johnson and Co.

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PLATES

PLATE I

FIG. 2. Photograph of two litter mates, age 202 days. The smaller animal, (pig 7-35), received the same basal diet and vitamin mixtures as the larger one (7-27) except that calcium pantothenate was omitted. Note not only its smaller size but the almost total absence of hair, especially over the rump. Pig 7-35 was ataxic at this time and suffered from diarrhea.

FIGS. 3 AND 4. Two views of a black pantothenic acid-deficient pig (7-60) at the ages of 106 and 119 days respectively, to show the extreme thinness of the hair.

FIG. 5. Control animal, age 119 days. Note the excellent coat of hair.

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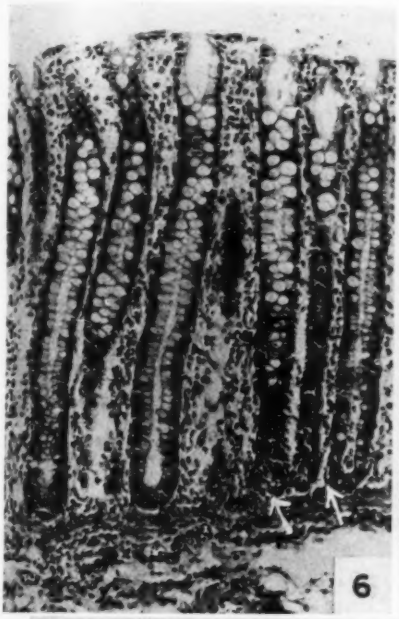
PLATE II

FIG. 6. Photomicrograph of colon (× 125) of pig 7-52. The glands at the left are lined by cells containing large mucus vacuoles and appear normal. There are relatively few cells in the periglandular tissue. At the right (arrow) some of the cells have lost their vacuoles and are swollen; leukocytes are seen in the lumen.

FIG. 7. Photomicrograph of colon (× 125) of pig 7-52. This is a field near that of figure 6. Note great increase in interstitial cellular infiltration and atrophy of glandular cells, especially the superficial ones.

FIG. 8. Photomicrograph of colon (× 125) of pig 7-37. There are some normally appearing glands, atrophic glands, and two with dilated lumens containing leukocytes. Their lining cells are flattened.



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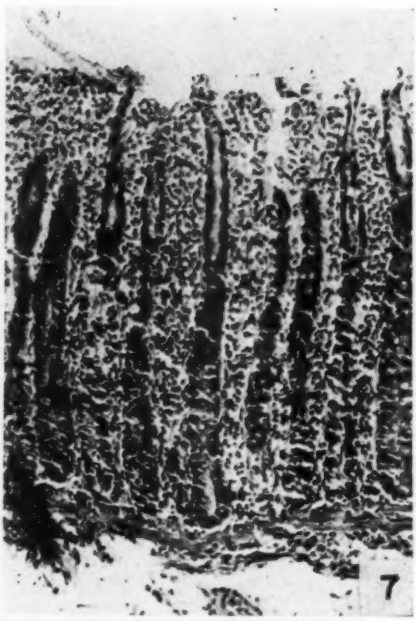

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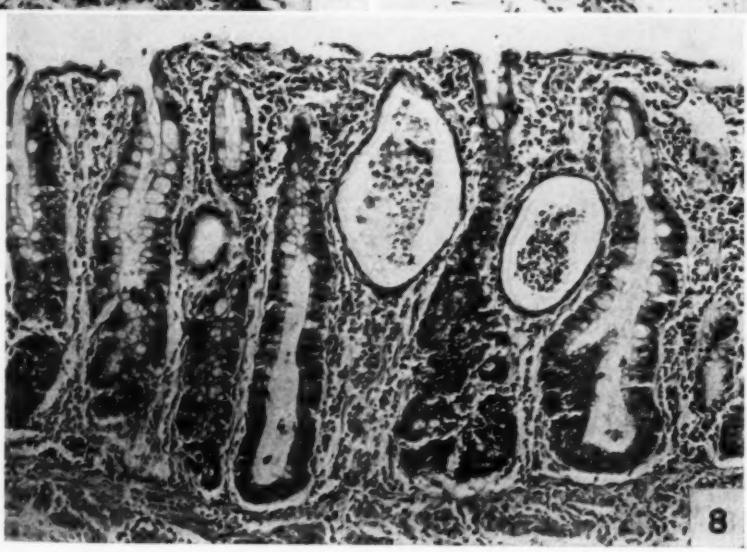

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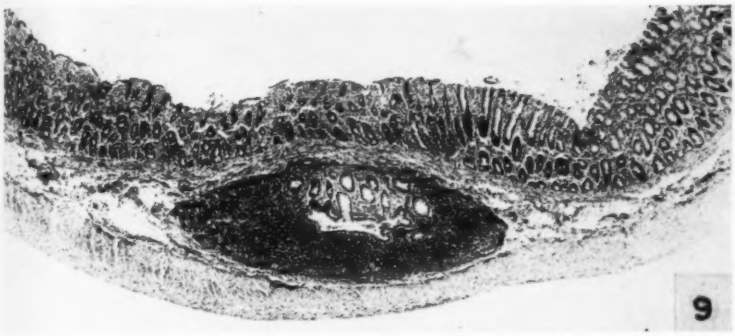
PLATE III

FIG. 9. Photomicrograph of colon (× 25) of pig 7-52 to include a solitary lymphoid follicle. Note prolongation of gland in center. Lumen contains a few leukocytes. Lymphoid tissue is slightly hyperplastic.

FIG. 10. Photomicrograph of colon (× 25) of pig 7-52 to show abscess formation in center of lymphoid follicle which is larger than that seen in figure 9.

FIG. 11. Photomicrograph of edge of ulcer (× 50) of colon of pig 7-17. Note cellular infiltration of submucosa beneath and exudate on surface.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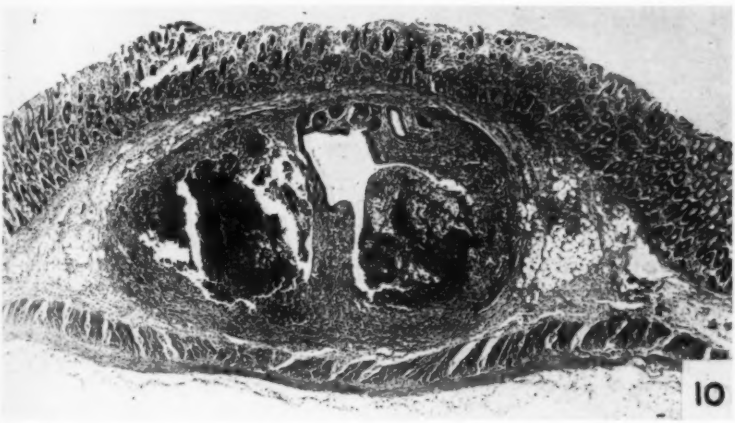
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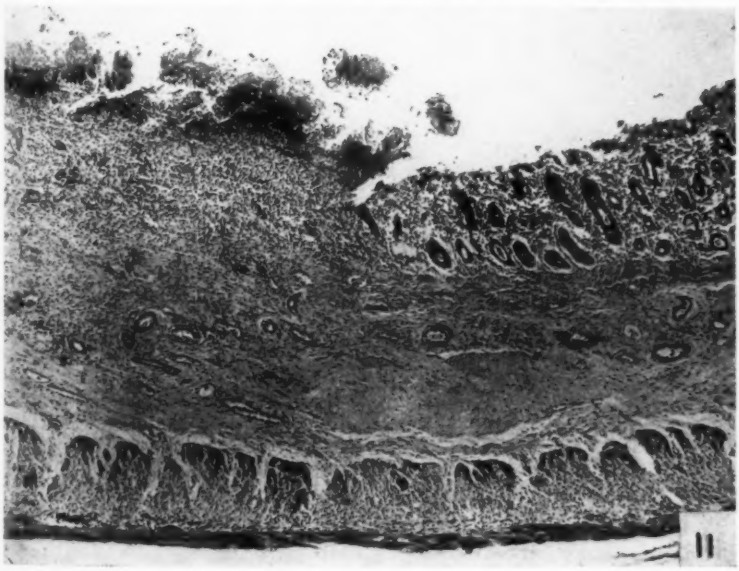
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PLATE IV

FIG. 12. Photomicrograph of colon (× 50) of pig 7-39. The mucosa is narrow, and there are relatively few glands. In the submucosa and throughout the entire wall there are chronic inflammatory cells. There is a great deal of scarring and almost complete absence of muscle fibers.

12

THE ANTIBACTERIAL PROPERTIES OF CRUDE PENICILLIN

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A new and promising field in the therapy of bacterial infections was opened by the important observations of Fleming in 1929 (1) on the antibacterial substance produced by a strain of the mold Penicillium. It was established at that time that the active substance was produced best in ordinary nutrient broth, at a temperature of 20° C., that it was filterable and non-toxic and had its most marked action against pyogenic cocci, whereas the coli-typhoid group, H. influenzae and Enterococci, were insensitive to it. Fleming employed the material chiefly as a differential medium for the purpose of isolating H. influenzae by inhibiting the Gram-positive organisms, but he suggested that it might be used as an antiseptic. In another article, this same author (2) stressed the necessity of adjusting the alkaline culture filtrate to pH 6.8 in order that it may remain potent. He also found that certain Gram-negative cocci—meningococci and gonococci—were sensitive to this substance which he called penicillin. It was then shown in 1932 by Clutterbuck, Lovell and Raistrick (3) that the antibacterial substance could be obtained by cultivation of this same strain of Penicillium on a synthetic medium. They employed the modified Czapek-Dox medium which contains 3 grams of sodium nitrate, smaller concentrations of other salts, and 40 grams of glucose to 1000 cc. of distilled water. They made the important observation that it could be stored and kept active at 0°C., extracted by ether at pH 2, and evaporated in vacuo at pH 5 to 6. They also found that it was readily inactivated by oxidation.

Since 1940 a great deal of interest has arisen concerning the chemotherapeutic possibilities of this substance and several reports dealing with methods of preparation, purification and clinical application have appeared from England (4, 6, 14, 15, 16, 17) and this country (5, 7, 8, 9, 10, 11, 12, 13, 18, 24, 25, 29). The most important observations

resulting from this work indicate that penicillin can be extracted from the crude culture filtrate; it is active mainly against Gram-positive cocci, but also affects gonococci, meningococci, Cl. welchii, anaerobic streptococci and other organisms; the extract is active in dilutions as high as 1 in 5 to 20 million; it is active in the presence of pus, exudates, tissue cells and p-aminobenzoic acid, and is non-toxic even in very large doses. In addition to these properties the penicillin has been found effective in protecting mice against staphylococcal, streptococcal and pneumococcal infections (4, 5, 10, 11, 13, 18, 19) and against experimental infections with Cl. welchii (4, 17). Two of these studies (13, 19) were carried out with sulfonamide-resistant pneumococci.

Preliminary reports of the clinical use of penicillin are likewise quite encouraging. However, there are certain drawbacks which put limitations on the practical application of the *in vitro* activity of penicillin. Its extraction is rather complicated and requires a great deal of crude material; the exact chemical structure of penicillin has not been determined; it is not very stable; it loses its activity in a few days or weeks if not kept cold and may be inactivated by bacterial contaminants; it is excreted rapidly by the kidneys when injected intravenously, and it is inactivated by gastric juices so that it cannot be given satisfactorily by mouth.

It seemed to us that if a potent antibacterial substance could be produced in a simple medium it would be important to study the properties of the crude penicillin in an attempt to obtain a filtrate that would be satisfactory mainly for local application. The following studies were carried out with that end in mind, realizing at the same time that the potency of the crude material would be less than one thousandth that of the extract and, therefore, that we would expect it to be entirely supplanted by the "pure" penicillin as soon as that becomes generally available.

MATERIALS AND METHODS

Penicillium Culture. The culture of penicillium used in most of this work was a subculture of the original strain of *P. notatum* of Fleming and was given to us by Dr. Edwin C. White. Other strains have been tried, one of which was found to be equally or more active than the original one, but the remainder either showed much less activity or none at all.

Media. The medium recommended by Dr. White was "Bacto-tryptone" (Difco) 20 grams, glucose 3 grams, NaCl 5 grams in 1000 cc. distilled water. The pH was adjusted to 6.8 and it was then autoclaved. In the medium the fungus showed a diffuse, rather fine, cotton-like sort of growth beneath the surface in one day; a white surface growth appeared in 3 days, which then coalesced and became green and ridged in about 5 days. After approximately 7 days the surface contained many deep yellow drops of fluid and at about this time the material was usually found to have its maximum antibacterial titer. The modified Czapek-Dox medium was also tried, both by using the standard ingredients and by substituting brown sugar for glucose (as suggested by Miss Clara McKee of the Squibb Institute); but although the fungus grew luxuriantly, potent antibacterial substance was not produced by our strains of penicillium in this way.

A medium simpler than the original "tryptone medium" was sought for in an effort to eliminate potential sensitizing substances or allergens as completely as possible. In this way, for clinical application, we might hope to have a solution less apt to produce local reactions due to sensitization and one that might possibly be used for intravenous administration. Consequently, we obtained from the Difco Laboratories two lots of chemicals, one called "Casamino Acids" and the other "Casamino Acids Technical". They are both described as completely hydrolyzed proteins made by the process of acid hydrolysis of casein. The chemical analysis of the "Casamino Acids" is given as: moisture 3.12 per cent; ash 16.36 per cent; sodium chloride 13.1 per cent; nitrogen 10.6 per cent; P as PO_4 1.64 per cent. That for "Casamino Acids Technical": moisture 4.86 per cent; ash 40.8 per cent; sodium chloride 36.17 per cent and nitrogen 7.41 per cent. Satisfactory culture media were then obtained by using either of these two lots in proportions of 3 per cent in distilled water with brown sugar 2 per cent, this solution then being adjusted to pH 3.5 to 4.5 and autoclaved. Most of the studies to be reported here were carried out with one or both of these latter media.

Culture Flasks and Cultural Methods. The most satisfactory type of culture flask is a flat one of 500 or 1000 cc. capacity and having an eccentric neck and mouth. Approximately 300 cc. of medium are put in the larger flasks and 200 cc. in the smaller ones. In this way a wide surface area is provided over a shallow medium of 1½ to 2 cm. in depth. This has been found by all those working in this field to be the most satisfactory depth of medium, for the penicillium in its growth secretes the active substance rather close to its surface growth and if the medium is too deep the antibacterial substance is, therefore, more dilute.

The flasks are seeded with a loopful of spores from stock cultures which are kept on solid media. It is important to be very careful to avoid any contamination either at the time of primary inoculation or when testing for antibacterial activity, as such contaminating organisms will usually either inhibit the growth of the mold or even destroy the penicillin that is being produced.

The character of the growth has been described earlier. After 4 or 5 days, if the surface growth is confluent and greenish, it is tested for changes in pH and development of antibacterial potency by pipetting out a few cc. under sterile precautions. The significance of pH changes will be discussed later.

Temperature of Cultivation and Other Environmental Factors. The mold grows best at "room" temperature, which obviously shows a rather wide variation depending on the season. We have found the most satisfactory temperature to be from 22° to 25°C. It shows no growth at incubator temperature, thus allowing for trial tests with bacterial cultures, without the necessity of filtration; this was originally pointed out by Fleming. We have ordinarily kept our culture in diffuse daylight close to a window, but have found that the potent penicillin is produced equally well if the mold is grown in the dark.

Filtration. The type of filter that yielded the best results in our hands was a Seitz, with a large pad and a funnel-shaped reservoir that would hold approximately 500 cc. During the early part of this work it was noted that a great deal or all potent material was removed by passing it through a Seitz pad, indicating that a certain amount of it is absorbed by the pad itself. However, the next batch to be passed through that filter pad will retain its activity, showing possibly that as the filter becomes saturated it no longer absorbs the penicillin. Our routine method has been to pass through about 500 cc. of some inactive discarded filtrate first. The contents of the culture flask are then emptied into the filter and the pH is tested roughly with nitrazine paper. (This test having been checked frequently before with the pH meter.) The pH is adjusted to approximately 6.5 to 7 and then the fluid is filtered. The resulting clear solution is usually bright yellow with a greenish tint, and is what we refer to as "crude penicillin". It is then put into a freezer at −30°C. and kept at this temperature, as we have found it to be preserved better under those conditions than any others. The influence of temperatures on its activity will be discussed more fully later.

Methods of Testing. Titration. The substrate of the growing penicillium or the filtrate is tested for its activity with stock cultures of staphylococci or streptococci or with various other types of bacteria which are encountered from time to time in routine cultures. Rough tests are used to determine whether or not the organism is sensitive to the penicillin, which is tested simultaneously with a strain that is known to be sensitive; more careful titrations are carried out to determine the exact potency of each lot of penicillin. The "rough tests" are done usually by adding the substrate or filtrate in amounts of 0.5, 0.1 and 0.05 cc. to 5 cc. of broth and inoculating this with 1 drop from a 1 cc. pipette of an 18 to 24 hour broth culture of the bacteria under investigation. In this way, we have dilutions of penicillin of approximately 1:10, 1:50 and 1:100. There is, of course, a tremendous variation in the number of organisms in the different inocula used, both because of the varying rates of growth of the bacteria and the variation in the actual size of the drops from the pipettes. However, others (6) have shown, and we have also found it to be true, that the antibacterial effect of this substance is relatively little affected by the number of organisms used, and so this convenient method of assay may be employed except when actual bacterial counts are required for special purposes. In making more exact titrations a row of seven tubes is set up with 9.5 cc. of broth in the first and 5 cc. in each of the others; 0.5 cc. of the penicillin is then added to the first tube, this is thoroughly mixed and 5 cc. transferred

to the second tube. The same procedure is repeated with the third tube and so on to the end. In this way serial dilutions are made of the penicillin from 1:20 to 1:1280. One tube of 5 cc. of broth is set up for the control. One drop of the culture is then added to each tube and they are incubated for 18 to 24 hours, or at times for 48 hours, and then read. The titer of "complete inhibition" is taken as the last tube which is entirely clear, and "partial inhibition" is designated to the tube in which there is growth but much less than in the control. Subcul-

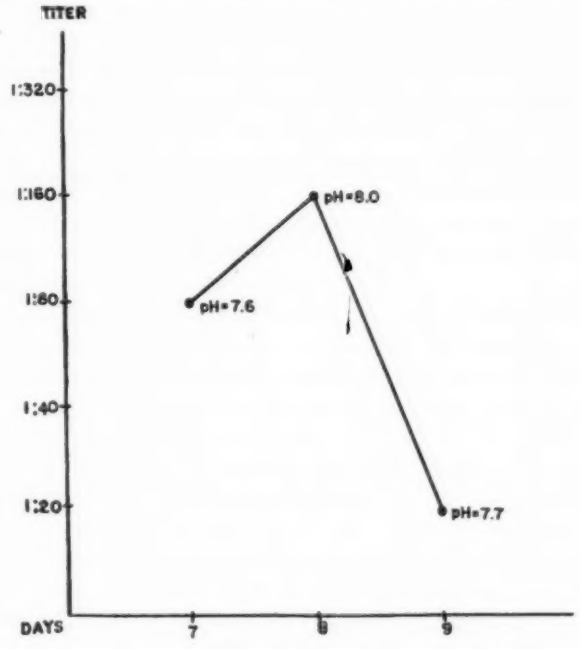

FIG. 1. CHANGES IN BACTERICIDAL TITER AND pH DURING GROWTH OF PENICILLIUM

tures are often made from these tubes on blood agar plates, and it is usually found that a few organisms are still viable in the tubes of the higher dilutions even though they appear clear, while the subcultures from those of the lower dilutionsremain sterile.

Changes in pH During Growth. The pH of the culture medium which starts at 3.5 to 4.5 remains at this approximate level for the first 3 or 4 days; then as the growth of the mold becomes more profuse and confluent the pH rises rapidly.

This is associated with the rapid development of antibacterial activity, which usually reaches its peak when the pH is between 7 and 7.5. It then continues to rise, going higher than 8 and then dropping again. As the pH approximates 8 and then begins to drop—which may require not more than 24 hours—the medium rapidly loses all antibacterial activity. It is very important, therefore, to filter the material and then adjust the pH to 6.5 to 7 before the penicillium has reached

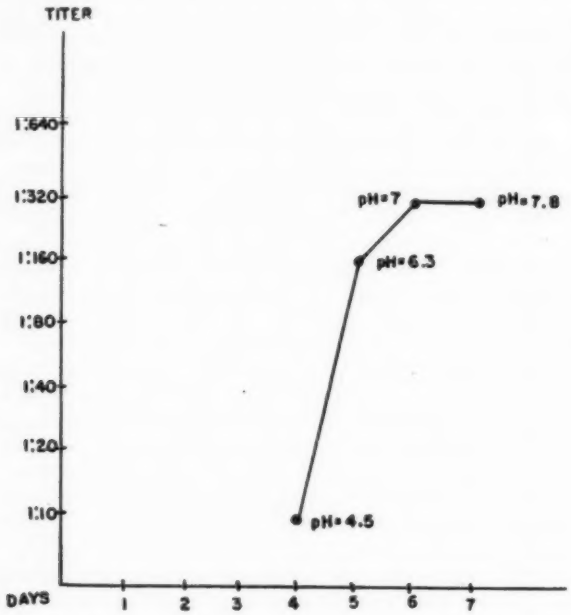

FIG. 2. DAILY BACTERICIDAL TITER OF PENICILLIN DURING GROWTH CORRELATED WITH CHANGES IN pH

the height of its alkalinity, as the most active and most stable penicillin will be obtained in that way.

The daily change in antibacterial titer during the growth of the penicillium associated with the changes in pH is illustrated by the following figures: The curve in Figure 1 is a graphic demonstration of the necessity of filtering the substance before the titer and the pH have started to fall. Figure 2 also shows daily correlation between rising titer and pH; a strain of *Staphylococcus aureus* being used as the test organism.

PROPERTIES OF THE CULTURE FILTRATE

Stability. Penicillin has been found to be very unstable; this holds true for both the crude filtrate and the purified extract, and represents a serious handicap in its use except where more or less ideal conditions can be maintained for its preservation. As for the crude penicillin, we have found that it loses its potency completely if kept at incubator temperature for 24 to 48 hours or at room temperature (22 –25°C.) for 4 to 6 days. At ice-box temperature it usually reamins active for approximately 10 days to 3 weeks, although there is some decrease in potency during that time. If the pH is adjusted to 6.5 to 7 and the penicillin is kept frozen in the low-temperature ice-box, it may be preserved regularly for 2 months, and has been kept active for as long as 5 months. Thawing and then refreezing does not seem to result in its deterioration if it is not kept out too long. As the penicillin deteriorates, the pH has been noted to rise; going from the original level of approximately 7 to above 8 (Figure 3).

We have also noted that penicillin will keep a little better under anaerobic or partially anaerobic conditions. For example: a lot of penicillin which showed complete inhibition of a test strain of staphylococcus to a titer of 1:160, was put into a test tube and the tube heated in order to drive off most of the air. The top of the cotton stopper was then flamed, and it was pushed down into the tube a short distance by a rubber stopper which was fitted into place, thus producing and maintining partially anaerobic conditions. This tube and another containing some of the same penicillin, but only the usual cotton stopper, were kept at room temperature for 5 days. At the end of that time the one in which partial anaerobiasis was maintained still showed a titer of 1:80 against the same organism, and its pH was 7.5. The material in the control tube, on the other hand, had lost all its activity in 4 days and its pH had risen to 8.2. However, it was soon apparent that the penicillin could not be kept potent merely by preventing its exposure to air; for it was found that keeping it in an anaerobe jar in the incubator for a week resulted in complete loss of its activity and that keeping it under a deep layer of sterile mineral oil did not help preserve it either at room or ice-box temperature. Pyrex tubes and flasks were then tried in the hope of eliminating the possible factor of absorption of alkali from the glass. The results of this were

negative. However, it was preserved much better at ice-box temperature if kept in a well-filled flask and tightly sealed with a rubber stopper. Various chemical substances and vitamin K were also tried but without the desired effect.

The importance of sterility was stressed earlier in discussing the cultural methods, and the same precautions must be observed for the

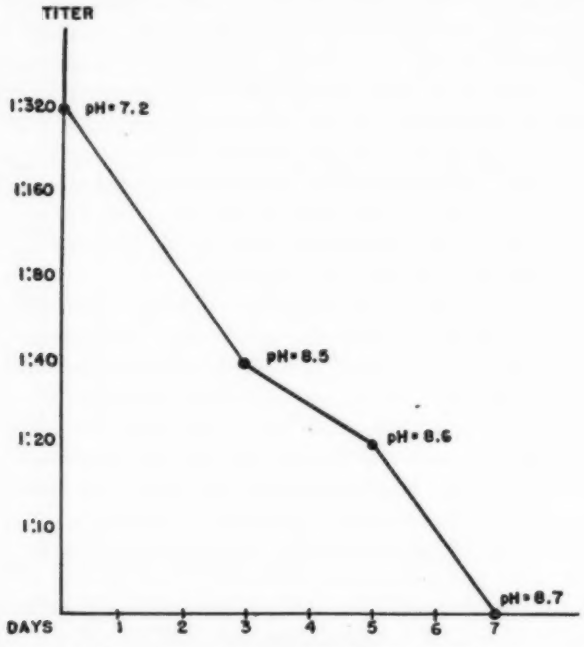

FIG. 3. EFFECT OF ROOM TEMPERATURE (22°–25°C.) ON BACTERICIDAL TITER OF PENICILLIN

filtrate. Many contaminating bacteria—especially Gram-negative bacilli *B. subtilis*, etc.—if allowed to grow in the penicillin for even a short time will destroy its activity. The most important points to be observed, therefore, in attempting to maintain the potency of the penicillin, seem to be to have its reaction close to neutral, to keep it frozen and to keept it completely free from contaminating organisms.

Heat Resistance. The crude penicillin has been found by us to be relatively heat-stable, whereas others (17) have found that the purified material loses most of its activity if kept at 100°C. for 30 minutes. This is demonstrated in Figure 4 in which the test organism is a *Staphylococcus aureus*. It is shown that the activity of penicillin which had a titer of 1:160 before heating dropped to 1:40 after heating for 1 hour at 100°C. The fact that it maintains this degree of potency, after being

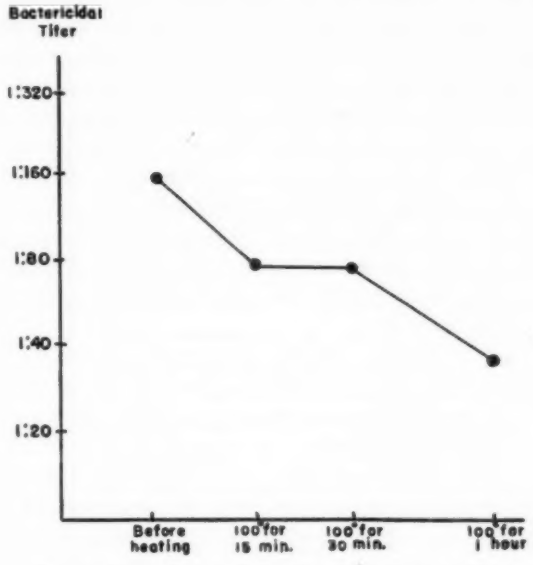

FIG. 4. RESISTANCE OF CRUDE PENICILLIN TO HEAT

in boiling water for 1 hour, would certainly indicate that it is rather heat-stable. Some of the material that had been subjected to this temperature was then put aside at room and ice-box temperature to see whether or not this heating—possibly by destroying some enzymatic activity—might aid in maintaining its stability. The results of this were negative, however.

Effect of Drying. Some of the penicillin was dried in vacuo from the frozen state by Dr. Margaret Smith at the Sydenham Hospital. This yellow powder went into solution in distilled water very readily, and

was redissolved in an amount equal to that of the original material. In this way, comparative tests could be run to see whether or not any potency was lost by drying. It was found in one test that the titer against a staphylococcus only dropped from 1:640 to 1:320 By redissolving another lot in 2 cc. of water the titer was increased again to 1:640. These methods have not been given extensive trials yet, but we may find that this is a better way to preserve the penicillin in an active state and also a way in which it can be concentrated to some extent. The dried material was found to be active to the same titer (1:320) after being kept in vacuo in the ice box for 2½ months.

Diffusibility. It has often been stated that penicillin is a very diffusible substance, and this property, no doubt, plays an important role in its use as an antibacterial agent. We also found this to be true, as it diffused readily through a cellophane membrane. This was shown by putting the crude penicillin inside a sterilized cellophane bag, tying this off and immersing it in tap water inside a sterile glass cylinder. This was then left in the ice-box overnight. The next day the fluids inside and outside the bag were about equally yellow and approximately equally bacteriostatic.

Effect of Pus, Blood, etc. Another important property that is mentioned in all discussions on penicillin concerns its lack of inhibition by pus, blood, exudates and para-aminobenzoic acid. This is a definite advantage over the sulfonamides. It has also been shown by employing the tissue culture method, that the activity of penicillin is not influenced either by living tissue cells or by the products of autolysis of cells (8, 9). We have been able to confirm these findings with pus, blood and para-aminobenzoic acid, and have repeatedly set up our experiments using the purulent material itself rather than the pure cultures of the organisms from the pus. The penicillin has been equally active under these conditions. The same hold true for both human and rabbit blood.

Influence of the Number of Organisms. Another rather unique characteristic of penicillin which has been pointed out before (6) is the fact that the number of organisms involved has relatively little effect on its antibacterial potency. Because of this, our titrations can be reasonably accurate although the size of the inocula varies tremendously. The 18 or 24 hour growth of staphylococcus in broth is a very heavy one

and the standard inoculum of one drop from a pipette into 5 cc. of broth has been found to result in a concentration of 3 to 16 million bacteria per cc. Although there is a great variation both in the size of the drops and the growth of the broth cultures, the titrations with a given lot of penicillin and a given strain of staphylococcus, nearly always come out to the same end-point. The same holds true for streptococci, the growth of which in plain broth or blood broth is not nearly as abundant as that of the staphylococcus, for the titers are approximately the same whether one drop or three drops are used. An actual example of this is provided by the following experiment: The titration of a lot of penicillin was tested against a 24 hour culture of staphylococcus using an inoculum in one test of 17 million organisms per cc. in the routine 5 cc. of broth, and in the other 8 billion organisms per cc. In each case there was complete inhibition at 1:40 and partial at 1:160. This type of reaction occurs over rather wide limits, but is not absolute, as some of the resistant strains of staphylococcus are found to be inhibited if the cultures are diluted 100 times before testing, and at the other extreme if the penicillin is added directly to fully grown cultures of staphylococcus—even in proportions of 1 to 1—sterilization is not accomplished. In this latter regard our conclusions are in agreement with those of Hobby et al. (10), who found that penicillin seemed to be effective only when active multiplication was taking place.

Varying Potency at Different Levels. It was noted that when the crude penicillin was frozen in the usual way and then allowed to melt, either by standing at room temperature or by heating, it would settle in more or less distinct layers. The lower layer would be quite dark, and if the flask was shaken gently, had a syrupy appearance. The top layer was quite light in color, and often if the process of freezing and thawing were repeated once or twice, the top layer became almost colorless, or a very light yellow. The middle portion showed a gradation in color between these two. In making tests from these different layers, we were interested to find that the penicillin from the lower layer was much more potent than that from the top. We thought, therefore, that by centrifuging, the most potent material could be separated more completely. Though it was tried several times, centrifugation merely served to mix the layers together again and the titer was then found to be equal throughout. The brown sugar in the medium is the

factor responsible for this phenomenon, and as it settles to the bottom after thawing it carries the greater part of the active penicillin down with it. This method seemed to us to offer a way of concentrating the penicillin, which could be done quite well by using long, flat-bottomed cylindrical flasks of approximately a liter's capacity. It was frequently noted that the lower portion was more than ten times stronger than the upper. The separation was somewhat more pronounced after the penicillin was frozen and thawed a second time, but repeated treatment of this sort did not change the titers at the different levels.

A rough separation of more active from less active material can also be effected by pouring off the fluid that forms during the first part of the thawing process. This fluid is much darker yellow and is also considerably stronger, though the difference is not as great as when the method mentioned above is employed.

Toxicity Experiments. One of the chief attributes of penicillin which many have reported (1, 4, 5, 6, 10, 18, 24, 25) is its relative lack of toxicity. Our studies with even the crude product confirm this, though in the animal experiments temperatures were not taken to determine its pyrogenic effect. (During the process of purification of penicillin the pyrogenic fraction is removed.) We felt that the media themselves should be relatively harmless to animals since the protein content was low and relatively simple, since its reaction was close to neutral and it was nearly isotonic. The following methods of administration to animals were employed: (1) as eye-drops to three rabbits on several occasions, both before and 10 days to 2 weeks after intravenous injections of the same substance. No signs of inflammation resulted. (2) Intracutaneously on the abdominal walls of mice in amounts as great as 0.5 cc. Intracutaneously in rabbits' ears both before intravenous injection and 10 days to 2 weeks afterwards. There were no signs of redness, edema nor inflammation. (3) Intraperitoneally into mice and guinea pigs. The doses in the mice were from 1 to 4 cc. and were repeated many times after intervals of weeks and months. In the guinea pigs the injections were of 5 cc. each and were likewise repeated after 1 and 2 weeks in order to determine the presence or absence of hypersensitivity. No reactions of any sort were ever noted in these animals. (4) Intrabronchially in 2 rats. This was done by Dr. Edward Irons. 0.5 cc. was injected into the left lower bronchus of

each animal while under anesthesia. The rats were then suspended in an upright position for 30 minutes and allowed to recover from the anesthetic. They remained perfectly well and when killed and autopsied 48 hours later, nothing abnormal was found grossly or microscopically. (5) Intravenously to 8 rabbits, 3 guinea pigs, 2 rats, 1 dog and 3 mice. The rabbits were given 10–20 cc. of the undiluted material rapidly—in less than 1 minute—and the dose was repeated at various intervals. In a typical example, 2 rabbits were given 20 cc. each in this manner and showed no immediate or delayed effect whatever. The same two animals were reinjected with 20 cc. in the same way 11 days later and again showed no immediate or late reaction. Two rabbits were reinjected several months afterward, with negative results. The guinea pigs were not tested as effectively because of the difficulty of intravenous injection, but 3 of these showed no reaction to 5 cc. One rat was given 1 cc. and another 2 cc. intravenously with no reaction. Three mice were injected in the tail vein with 1 to 2 cc. The injection was repeated in two of them, 11 days later. None developed a reaction of any sort, and all 3 mice were tested at the same time intradermally with completely negative results. Only one injection was done in a dog, which received 25 cc. undiluted in a leg vein. It was given rapidly and produced no noticeable effect. From these experiments, therefore, we must conclude that this crude penicillin is neither toxic nor irritating to the tissues of the test animals. Also in these limited tests it has not seemed to produce hypersensitivity, though, of course, this does not mean that the same will hold true for human beings.

Cyto-toxicity of Penicillin. Dr. George Gey was kind enough to try the effect of this same substance on growing tissue cells in tissue culture, in an effort to determine its degree of toxicity to isolated cells. His findings were as follows: "Fresh filtrates and stored (−32°C. for 2 weeks to 2 months) filtrates of penicillin culture are not toxic when added to tissue cultures of human and rat normal and malignant cells. Continuous cultures containing 10 per cent of penicillin were without toxic effect after more than 2 weeks exposure. Exposure to pure undiluted filtrate for 24 hours also does not kill tissue-cultured cells. Longer periods of exposure were not tried."

Organisms Tested. In vitro tests have been made on a number of

organisms, the figures for which are given in Table I. More than half of the staphylococci, streptococci, and pneumococci were tested by the usual serial dilution method by Miss M. Kathleen Foley in connection with some studies being carried out with Dr. S. J. Crowe on the bacteriology of chronic infections of the ear, nose and throat, and their treatment with tyrothricin and penicillin. The results of these studies

TABLE 1

Organisms Tested with Crude Penicillin

SENSITIVE	NUMBER OF STRAINS	DEGREE OF SENSITIVITY*	RESISTANT	NUMBER OF STRAINS
Staphylococcus (aureus and albus)	90	+-++++	Staphylococcus aureus	12
β-hemolytic streptococcus	40	+++-++++	β-hemolytic streptococcus†	1
α-hemolytic streptococcus	11	+++-++++	α-hemolytic streptococcus†	2
pneumococcus	23	+++-++++	pneumococcus†	1
anaerobic streptococcus	10	+++-++++	Esch. coli	5
β-hemolytic streptococcus, Lancefield Group B	3	++-+++	α-streptococcus fecalis	5
β-hemolytic streptococcus, Lancefield Group C	4	++-+++	Proteus	3
Neisseria intracellularis (meningococci)	3	+++	Pseudomonas aeruginosa (pyocyaneus)	3
Neisseria gonorrhoeae	3	+++	E. typhosa	2
Cl. welchii	3	+-+++	H. influenzae	2
Actinomyces bovis	2	+++	Cl. tetani	1
C. diphtheriae	2	+		

* The range of sensitivity indicated as + to ++++ is a rough approximation of titers from 1:20 to 1:1280.

† These resistant strains were isolated and tested by Miss Foley.

will be the subject of a separate report (27). By far the greatest number of organisms tested were staphylococci, and it is interesting to note that there is a wide variation in the susceptibility of the various strains as regards their sensitivity to the action of penicillin. For instance, we found that many staphylococci were inhibited by our crude penicillin in dilutions as high as 1:1280, whereas others were only moderately sensitive—the titer being 1:160 or 1:320—and still others were rather resistant, being affected only in dilutions as low as 1:20.

The Penicillin-Resistant Staphylococci. We were particularly interested to find every now and then a strain of *Staphylococcus aureus* that was completely resistant to penicillin. In all, 12 such strains were encountered in the course of these experiments, and two of them were studied in more detail; the results were negative as far as establishing an explanation for these findings. The colonies did not show more than the average amount of hemolysis on blood agar, the organisms fermented mannite just as penicillin-sensitive controls did, and they did not produce capsules even when grown on blood with 10 per cent sterile milk in the medium (4 strains were tested in this way and compared with controls). Four of the strains coagulated human plasma—the same being true with four controls—and one of the resistant strains was slowly fibrinolytic. They were not toxin producers, whereas one of the two controls did produce toxin which was lethal to a rabbit—these tests were performed by Dr. Earl Burky of the Wilmer Ophthalmological Institute. The bacteria-free Seitz filtrates of these two resistant Staphylococci did not inhibit the antibacterial action of penicillin when added to the standard test media in proportions as high as 1 cc. of this filtrate to 0.2 cc. or 0.1 cc. of penicillin in 5 cc. of broth. Similar negative results were obtained when these same organisms were killed by heat, centrifuged, suspended in saline and then added to the test media as above. Ten of these strains were tested by Miss Foley with tyrothricin and two were found resistant to that agent. Three were also tested with sulfathiazole using three of the penicillin-sensitive organisms as controls; these were inhibited by the sulfathiazole, but were much more resistant to its action than 2 of the controls, high concentrations of the drug in a dilute broth medium being necessary for bacteriostasis. The third control organism, however, which was equally sensitive to penicillin showed the same relative resistance to the sulfathiazole. A report by Rammelkamp and Maxon (20) is interesting in this regard. They found as Abraham et al. (6) had also noted, that they could increase the resistance of *Staphylococcus aureus* by growing the organisms in increasing concentrations of the penicillin. Four of 14 strains of staphylococci which they isolated from patients with localized infections developed increased resistance to the penicillin during the course of treatment.

In summary we must say that the cause of this interesting variation

in the usual reaction of staphylococci to penicillin is not made clear by these studies. It is apparently a function of the growing bacteria. They were not found to produce a neutralizing or inhibiting substance in the media.

Streptococci. Most of the streptococci tested were obtained in cultures from throats, ears, blood, *etc.* and the greatest number of these belonged presumably to the *Lancefield Group A* of *beta-hemolytic streptococcus*, though they were not all grouped. The titrations were made in the usual way with plain broth, or more often, with 1 drop of rabbit's blood in the broth. Because the growth of streptococci is so much less abundant than that of staphylococci in these media a larger inoculum (0.1 cc.) was usually employed. All but one of this group of thirty-five strains were quite sensitive to the action of penicillin, the majority being killed in dilutions of 1:320 or 1:640 and inhibited at 1:1280. One other factor has to be taken into consideration, namely, the fact that staphylococci grow so luxuriantly in these media, whereas the growth of streptococcus is relatively sparse. Thus it is natural to presume that the staphylococci will be much harder to kill or inhibit under these circumstances than the streptococci, their growth requirements being so much less strict. We have no reason to presume, however, that the results of these in vitro tests can be interpreted as meaning that hemolytic streptococcal infections will be found more susceptible than staphylococcal infections to the action of penicillin in the body; under the latter circumstances the conditions for growth of the streptococci may be better, and because of this they may be more difficult to kill *in vivo* than in the test tube.

Four strains of *beta-hemolytic streptococcus Lancefield Group B* were likewise susceptible to penicillin. This is in contrast to their sulfonamide-resistance and may indicate another use for penicillin in the rare cases of infection due to these organisms.

Ten *anaerobic* or *microaerophilic streptococci* were studied and all were penicillin-sensitive, the bactericidal effect being found in dilutions as high as 1:640 and 1:1280. The titrations were made in the usual way, using Brewer's anaerobic liquid agar medium instead of broth. Two of the strains were beta-hemolytic; one being obtained from a blood culture and the other from a lung abscess. One other was obtained from a case of empyema, another from a case of bronchiectasis and most

of the rest from sinus infections. These anaerobic streptococci are representative of another group of organisms which are not susceptible to the action of the sulfonamides. The fact that they are sensitive to penicillin suggests that here we will find another rather large field of usefulness for this substance.

Three *beta-hemolytic streptococci Lancefield Group C.* were obtained from suppurating cervical glands of guinea pigs, and one from a patient with widespread deep ulceration of the skin. These were all killed or inhibited by penicillin, but in lower dilutions than those of Lance field Group A.

Eleven *alpha-hemolytic streptococci* were encountered mainly in routine cultures from the nose, throat, sputum or blood. All but two were killed or inhibited by penicillin, the dilutions being approximately the same as for the beta-hemolytic variety.

The *alpha Streptococcus fecalis* was found to be completely resistant. Five strains were used, four of which were isolated from cultures of urine, and the other from a post-operative case of empyema.

Pneumococci. Twenty-three different strains of these were isolated from the usual sources, and were titrated like the streptococci in the presence of a small amount of rabbit's blood. All but one were affected by penicillin in high dilutions.

Gonococci. Our experience with these organisms is limited to three strains, each of which had been found by Dr. Herbert Koteen to be resistant to the sulfonamides. Their growth in the medium employed was rather sparse in the control tubes, but each was killed at dilutions of penicillin of 1:100.

Three strains of *Cl. welchii* were found penicillin-sensitive; two of these at titers of only 1:50, but the other at 1:640.

Two strains each of *meningococci*, *C. diphtheriae*, and *Actinomyces bovis* were tested. The meningococci and actinomyces were affected in dilutions of 1:100, but the diphtheria bacilli only at 1:15. One strain of *H. influenzae* was inhibited in dilutions of 1:50 and two others were resistant. Three strains of *E. typhosa* were insensitive to this routine preparation of penicillin, but a slightly different type of penicillium colony was found growing with our stock culture and this, when grown on the modified Czapek-Dox medium, produced a substance inhibitory to two strains of *E. typhosa* in low dilutions. Similar results were

obtained with a culture of penicillium which was very kindly sent to us by Dr. Kocholaty who has described the isolation of "penatin" from the growth of this mold (24). Penatin, he finds, is active against *E. typhosa* in high dilutions as well as against the other usual organisms which are sensitive to penicillin.

Five strains of *Esch. coli*, three of *proteus*, two *of pyocyaneus* and one of the *para-influenza* bacillus, *B. alkaligenes* and *Salmonella suipestifer* were completely resistant to the antibacterial action of penicillin. Two strains of Gaffkya (micrococcus) tetragenes were studied. These were encapsulted organisms, and we were interested in finding out whether or not the capsule might protect the bacteria from the action of the penicillin. One of these was resistant and the other very sensitive to the penicillin.

Antibacterial Potency Measured in Oxford Units. An "Oxford" (or "Florey") unit is defined as that amount of penicillin which when added to 50 cc. of broth will just inhibit the standard inoculum of a test strain of staphylococcus. One method of obtaining this arbitrary measurment (6) is to place a small open cylindrical glass tube on a plain agar plate which has been covered by a fresh broth culture of staphylococcus. This cylinder is then filled with the penicillin under investigation and the agar plate incubated. As the penicillin diffuses out over the agar a zone of growth inhibition results which leaves a circle around the cylinder. The diameter of this circle is then determined and the measurement compared with that produced by standard penicillin solution of known strength. The standards being calculated in units per cc. allows the determination of the same figures for the solution under investigation. Using this method on several occasions with two of our test strains of staphylococci, we found that the figures for the unfiltered penicillin were from 20 to 40 units per cc. and for the material concentrated by freezing and thawing was 42 to 50 units per cc. On another occasion and using another strain of *Staphylococcus aureus* the readings were 40 units for the undiluted penicillin, 40 units when diluted 1:5, and 33 units at 1:10. Thus the results were almost the same, though the penicillin was diluted five and ten times. Using the test strain of staphylococcus obtained from the Squibb Institute—the "Heatley" strain—our figures were 45 units per cc. for the more concentrated material when used full-strength; 35 units per cc. when diluted 1:5; and 30 units at 1:10.

Unless this method of measurement is used continually so that one can become proficient enough to keep the conditions well controlled it seems to us that it is less reliable than the titrations in broth. Variables to be controlled include the degree of dryness of the agar, the sensitivity of the particular strain of staphylococcus being used and the fit of the glass cylinder to the agar to allow for a constant degree of diffusion. The finding of a very little difference in many of these tests, between the zone of inhibition produced by the full-strength penicillin and that which had been diluted, also serves to point out another inaccuracy in this test for the crude penicillin, but it is of interest from the standpoint of diffusion, as it indicates that we may get better diffusion at dilutions of 1 to 5 or 1 to 10 as well as satisfactory antibacterial results.

When compared with the standard by means of the broth titration method, the figures were lower, being approximately 12 units for the unfiltered material and 25 to 40 units per cc. for the more concentrated penicillin. These figures are certainly more accurate than the ones obtained by the cylinder method, because of the potential inaccuracies in the latter, as mentioned above.

MODE OF ACTION

1. Gross Appearance of the Titration in Broth. In testing a sensitive organism in the routine way and then examining the tubes the next day, we find the tubes in the lower dilutions quite clear, and then the next two will usually show slight and increasing cloudiness and the others will show heavy growth, almost as great as the control. If subcultures are now made on blood agar, we usually find the first two or three sterile, then one or two which, though clear grossly, show a few colonies, and then a heavy growth from the tubes which showed inhibition grossly. This is particularly true of staphylococci, in which the bactericidal effect is limited to the stronger dilutions and the bacteriostatic effect takes place in the next two or three dilutions. With streptococci and pneumococci, however, the bactericidal effect is almost always apparent at much higher dilutions. It has also often been noticed that if a row of tubes is set up in the regular way to test a strain of staphylococcus, and then incubated, the first 2 to 5 tubes—depending, of course, on the strength of the penicillin—remain clear throughout. The next tube will become quite cloudy in 6 to 10 hours

—though not as cloudy as the control—and then the next day will be almost clear; subculture of this tube shows a good growth. At this dilution, therefore, we find slight inhibition of growth in the early stages with sufficient bactericidal activity after further action to render the media definitely clearer, but without the ability to destroy all the bacteria.

2. Fate of the Bacterial Cell. It has been stated that penicillin does not kill hemolytic streptococci by lysis (10). In considering the above observations in which there was partial clearing of a moderately heavy suspension of bacteria, one wonders if lysis may not occur to some extent in these cultures of staphylococci. More direct observations were made on the bacterial cells in the following experiment. One cc. of a 24 hour culture of beta-hemolytic streptococcus was added to 5 cc. of broth and 0.5 cc. of penicillin in a centrifuge tube; the resulting suspension was slightly cloudy. A control tube without the penicillin was also set up. These were incubated and then spun down after 4 hours. A drop of the sediment of each tube was stained with methylene blue—without fixing—and examined microscopically. The bacteria which had been exposed to the penicillin were more irregularly stained and were slightly less distinct than the controls but did not show much change at this stage, and subcultures showed that they had not yet been killed. These tubes were reincubated and then examined again the next morning. At this time the control showed a profuse growth and the other was clear. Microscopic examination of the control showed normal appearing chains and clumps of streptococci which were stained rather deeply and whose outlines were clear-cut. Those from the tube with penicillin, however, were lightly and unevenly stained; their chains were less well-formed and their outlines much less distinct. Subculture from this tube was sterile. The same experiments were done several times with staphylococci using 0.5 cc., and no noteworthy change was made out in the cells during the period of inhibition. After 18 hours the culture was sterile, and the media which had been cloudy upon addition of the staphylococcus suspension, had become entirely clear; the sediment at this time had the appearance of deep staining granular debris in which there were no recognizable cocci. The control showed normal live staphylococci (which lost their blue stain in about 30 minutes, due to reduction of the methylene blue).

These experiments are of interest in relation to the observations of Gardner (22), who noted marked swelling and distortion of the bacteria during the inhibition by penicillin of their growth. Smith and Hay (23) observed similar morphological changes in staphylococci, during inhibition, and this swelling of the organisms was followed by lysis. These phenomena were associated only with the active growth of the staphylococci, for fully grown suspensions did not show such changes even with high concentrations of penicillin. The authors conclude that penicillin either acts directly on the cell wall of the bacterium or prevents the assimilation of certain growth factors necessary for fission of the growing cell. Our own experiments, though very limited, support the conclusion that beta-hemolytic streptococci are killed by penicillin without lysis, but that the cell of the staphylococcus is actually broken up under the influence of this agent.

3. Growth and Bactericidal Curves. The mechanism of the antibacterial action could be demonstrated by making dilutions and pouring agar plates at various intervals and with different dilutions of penicillin, after inoculating the tubes with staphylococci or streptococci. A control tube was used in most of the experiments to show the normal growth curve. In this way, composite charts could be constructed which demonstrate the bactericidal effects at the lower dilutions, the inhibitory effects at the higher ones. These could be compared with the normal curves of growth. The routine method employed was as follows: Serial dilutions were made with penicillin in broth—one drop of rabbit's blood being added to each tube when streptococci were tested —the dilutions ranging from 1:10 or 1:20 to 1:1280, and one tube containing 5 cc. of broth for the control. To each of the tubes one drop from a 1 cc. pipette of an 18 to 24 hour culture of staphylococcus or 0.1 cc. of a similar *Group A beta-hemolytic streptococcus* culture was added. Then, knowing the approximate strength of the penicillin, the dilutions chosen for the experiment were those which we would expect to show the phenomena mentioned above. From these tubes and the controls, the suspensions of bacteria were diluted in broth to such a degree as had already been found suitable for bacterial counts. Plates were then poured from them. The usual dilution factors for the staphylococcus controls were 1:100,000 for the first 2 hours, then 1:200,000 and as growth becomes profuse 1:1,000,000. For the tubes with staphylo-

cocci plus penicillin the one-hour dilutions were either 1:100,000 or 1:20,000; as killing took place the dilutions were reduced to 1:10,000 and finally 1:1000. The same general scheme was followed with the streptococci, except that the reduction in dilutions was made earlier,

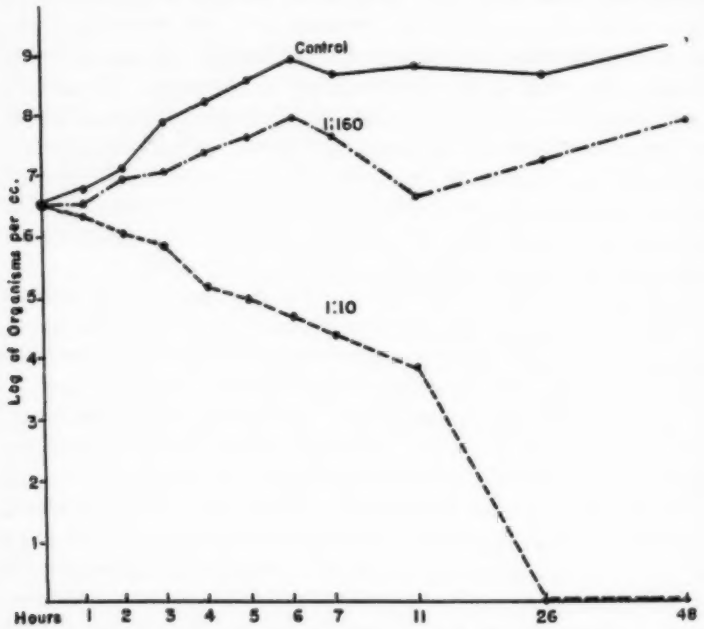

FIG. 5. EFFECT OF TWO DILUTIONS OF PENICILLIN ON STAPHYLOCOCCUS AUREUS (H) COMPARED WITH NORMAL GROWTH CURVE

Dilutions of penicillin: 1:10 = — — —, 1:160 = —·—, control = ———. This demonstrates the continuous bactericidal action in the lower dilution and only slight inhibition in the higher.

as these organisms were killed more rapidly. Plain agar was used for the staphylococcus plates and blood agar for the streptococcus. In this way, a plate was poured for the control before incubation, and then from the "test" tubes and the control at intervals of one hour or longer. The colonies were counted after 24 and 48 hours incubation. The results are demonstrated by the curves shown in Figures 5 to 8.

These charts show, first of all, that the speed and degree of action of the penicillin depend on its strength. For instance, in Figure 5 we find that its bactericidal action is rather slow and though growth of the

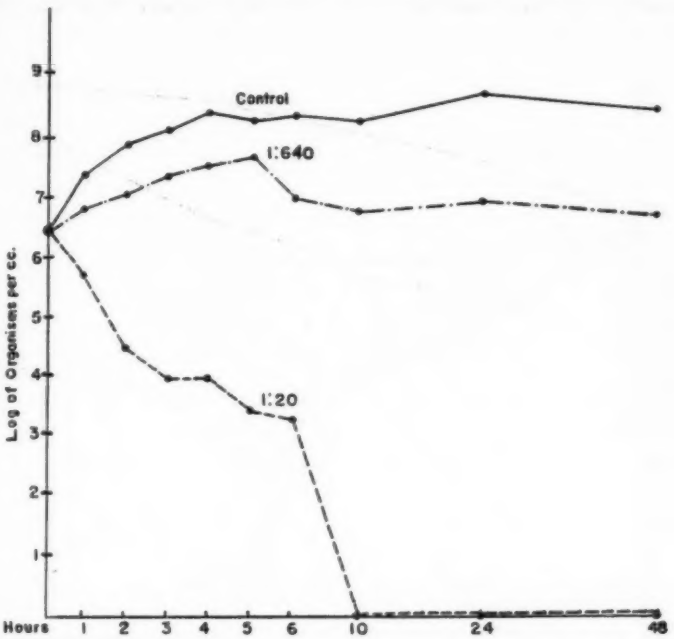

FIG. 6. BACTERICIDAL AND INHIBITORY EFFECT OF TWO DILUTIONS OF PENICILLIN ON BETA-HEMOLYTIC STREPTOCOCCUS (J); WITH CONTROL GROWTH CURVE

Dilutions of penicillin: 1:20 = — — —, 1:640 = —·—, control = ———. There is a marked reduction in the number of organisms in the first two hours in the lower dilution with sterility after ten hours, but only slight inhibition in the higher dilution.

staphylococcus is inhibited, the killing effect is not apparent to any great extent until 2 to 4 hours. In Figure 6, on the other hand, we find a marked bactericidal effect in the first hour in the case of the streptococcus then more gradual but steady killing during the next five hours, and finally sterility at ten hours, this sterility being maintained through

48 hours. Other points of interest to be gathered from these bactericidal and growth curves and demonstrated in Figures 7 and 8 are found in the curves showing, at first, very slow inhibition in the middle dilutions of penicillin and then the killing effect after six to nine hours. At

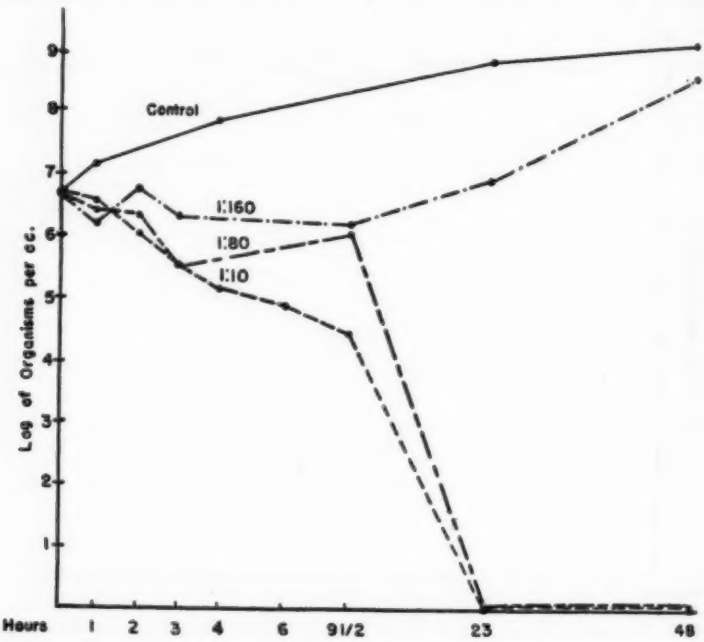

FIG. 7. EFFECT OF PENICILLIN IN VARIOUS DILUTIONS ON STAPHYLOCOCCUS AUREUS (D)

Dilutions of penicillin: 1:10 = – – –, 1:80 = — — —, 1:160 = —·—, control = ———. (No points charted for 1:80 and 1:160 at 4 and 6 hours as dilutions used were too high.)

the higher dilutions the bactericidal action is even slower and less complete; then, as there are a few organisms left alive and the dilute penicillin loses its activity at incubator temperature, there is a regrowth of the bacteria at 24 or 48 hours. While it has been claimed that the action of penicillin is bacteriostatic, our observations are in agreement with those who find that it is also bactericidal.

These curves also serve to point out a distinct difference in the action of penicillin as compared with both tyrothricin and the sulfonamides. In the case of the tyrothricin the bactericidal action takes place almost immediately. Plates that were poured within the first 15 minutes showed complete sterility when a test strain of beta-hemolytic strepto-

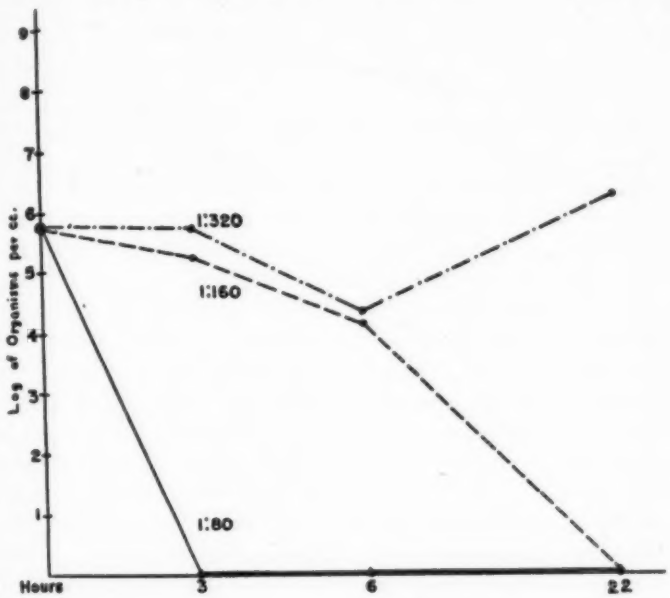

FIG. 8. BACTERICIDAL AND INHIBITORY ACTION OF THREE DILUTIONS OF PENICILLIN ON BETA-HEMOLYTIC STREPTOCOCCUS (GROUP A)

Dilutions of penicillin: 1:80 = ———, 1:160 = — — —, 1:320 = —·—. This again shows the more rapid bactericidal action in the lower dilution, the slower effect at 1:160 and only slight inhibition in the highest dilution.

coccus was employed. The same type of reaction was noted when a staphylococcus culture was tested with tyrothricin, the subculture being almost completely sterile in 30 minutes and entirely sterile from 2 hours on. The character of the antibacterial action of the sulfonamides is well-known; it is mainly bacteriostatic and only bactericidal in strong solutions, if at all. Hobby and coworkers (10) have demon-

strated similar features in these three classes of antibacterial agents. Their results with penicillin were much like ours, though the curves do not show just how soon the staphylococci are killed, as the counts were made at much longer intervals. Another finding of interest in their experiments was that the addition of blood to the test medium enhanced rather than retarded the speed at which their test strain of streptococcus was killed by penicillin. They found that the penicillin was not destroyed or removed from solution in any detectable amounts by the bacteria.

It is important to determine the rapidity of action of an antibacterial substance such as this; for if we plan to use it in the local treatment of infections we would like to know whether or not it can be kept in contact with the infected tissues or bacteria and in adequate concentration for a long enough time to insure its effect. From these *in vitro* experiments we can definitely say that a dilution of 1:20 or even 1:160 shows a moderate bactericidal action in one hour and a continuing effect of this sort for 12 or 24 hours. Therefore, assuming that the reaction of the penicillin in the tissues simulates that in the test tube—and we have reason to expect this, inasmuch as it is not inhibited by pus, blood, etc.—and assuming that it is used full-strength or in dilutions of 1:1 to 1:5, we can allow for its dilution many times over by tissue juices, exudates, etc. and still expect it to kill the susceptible bacteria. We have found, in studies with Dr. Crowe, that this reaction does actually occur in the tissues. Many times in sinuses chronically infected with organisms susceptible to penicillin, sterile cultures have been obtained after one or two applications.

POSSIBLE CLINICAL APPLICATION OF CRUDE PENICILLIN

As mentioned previously, the field for this type of therapy will be that of local application. The problem is, of course, to bring penicillin into as close contact with the infected tissues as possible, and to repeat this application at frequent intervals. The fact that it is not irritating enough to cause coagulation of proteins which might protect the bacteria beneath the surface and that it is very diffusible suggests that it may serve a very useful purpose in some chronic or acute local infections. We have not had any very extensive experience as yet, except in sinus infections. Penicillin has been employed in many of these

cases by Dr. Crowe and others now for over a year, and a summary of their results as well as the bacteriological observations made in these cases by Miss Foley are being published separately. They have had encouraging results in many cases in which the bacteria were found to be sensitive by *in vitro* tests to the action of penicillin. The problem is a difficult one, for in these patients there is often a great deal of chronically infected granulation tissue, and the difficulties in sterilizing such tissues are obvious. If complete sterilization is not accomplished, the few remaining bacteria might be expected to multiply again, as phagocytosis may not be adquate in such lesions. The aim in these conditions would, therefore, seem to be to treat repeatedly, until the bacteria can be killed or inhibited long enough for the body's natural mechanisms of defense to become adequate to protect against continued low grade infection or reinfection. The same principles hold true in treating infections of the throat.

We have had a few encouraging preliminary results with the spraying of throats and the nasal passages in Group A streptococcus carriers, but there have also been complete failures. In the latter the patients usually were found to have infected tonsils in which case one could not hope to rid them of the deep-seated infection with local therapy, and tonsillectomy was necessary. This field is worthy of further investigation, as in patients in whom there are not deep infections in the tonsils, we would expect good results if the spray were used often enough and in such a way as to come in contact with the bacteria themselves.

Local treatment with the crude penicillin was tried in nine patients with chronic leg ulcers, with negative results. These were all ulcers of long standing, most of them associated with circulatory disturbance and surrounded by thick borders of granulation and fibrous tissue. *Staphylococcus aureus* was isolated from most of them, but some also were infected with alpha or beta-hemolytic streptococci, and in several proteus or pyocyaneus were found. The treatments were given from 1 to 3 times a week and in the form of wet compresses which were left on. Most of the patients showed some improvement clinically, but no more than one might expect from the increase in frequency of the dressings, and none showed definite bacteriological improvement, two of them even developing secondary infection with pyocyaneus or

proteus during the course of the treatment. This type of therapy would not be expected to be effective in such conditions, because the penicillin loses its effect in the course of a day or so and then may serve as a culture medium for any bacteria that are left, and it is useless in the presence of proteus or pyocyaneus. The only way in which we could hope for successful treatment of these chronic leg ulcers with crude penicillin would be to have the patients in bed and the material sprayed over the ulcer every 2 or 3 hours. One would, of course, expect better results in ulcers of shorter duration or in recently infected wounds.

Local therapy with penicillin, either in the crude form, or when it is available, with the purified material, might be expected to be of benefit in many different types of localized infections due to the pyogenic cocci, anaerobic streptococci and possibly gonococci and meningococci. It would also seem well worth trying in cases of meningococcus carriers. A recent report by Herrell and others (28) is interesting as regards the effect of penicillin on gonococci. They gave the purified substance by intravenous drip to three patients with sulfonamide-resistant gonorrhea, with rapid cure in all three.

DISCUSSION

The growth and preparation of crude penicillin is relatively simple and can be carried out in any bacteriological laboratory. The chief drawback to its use is its instability; however, it can be preserved for long periods if neutralized and kept frozen. It is also relatively stable at ice-box temperature where it usually remains active for several weeks if kept in rubber-stoppered bottles. It should not be left at room temperature for more than a few hours at a time and between treatments it is best to replace it in the ice-box or to refreeze it if the intervals between use are very long. We have found, in using the medium prepared with brown sugar, that by freezing and thawing the penicillin, it can be recovered from the lower portion of the container in a concentration which is sometimes 10 times as great as that of the top.

The most satisfactory way in which to test the potency of the penicillin in any infection is either to isolate the organism responsible for the infection and then determine the degree of its inhibition in liquid

media by the penicillin, or to make similar tests with the whole culture just as it is received. In preliminary trials with cultures received mainly from chronic sinus infections it seems that this method may offer an accurate guide to its usefulness in treating these infections, as the *in vitro* activity appears to parallel its effect in the tissues to a considerable extent.

The crude penicillin is not toxic to animals when administered in large amounts either intravenously, subcutaneously, or intracutaneously; nor are there signs of local irritation of tissues or of development of hypersensitivity in these animals. It also was found to have very little untoward effect on growing tissue cells. In patients no local discomfort was produced by its application to ulcers nor by spraying in throats, but marked irritation sometimes resulted from instillation in the nose. This is much less marked, however, if it is diluted from one to three times with normal saline.

The penicillin exerted its most marked antibacterial effect on streptococci (all except the *alpha-Streptococcus fecalis*), pneumococci, anaerobic streptococci, meningococci and staphylococci. More of the latter were studied than any others and about 13 per cent of them were resistant to the penicillin. The reason for this resistance is not clear. It is dependent to a slight extent on the number of organisms present, for when very few are used in the test their growth can be inhibited. Other resistant organisms included *Esch. coli*, proteus and pyocyaneus. *E. typhosa* was resistant to the regular penicillin but young cultures of two new strains of penicillium on synthetic medium were effective against this organism in low dilutions. Contamination of the media during growth, or of the active filtrate by any of these organisms or by *B. subtilis* may completely inactivate penicillin.

The crude penicillin was only moderately active against two strains of *Cl. welchii*, but quite active against a third one; others have found this organism quite sensitive. As mentioned previously there have been reports (4, 17) of the successful use of penicillin in experimental *Cl. welchii* infections.

The organisms are killed in the lower dilutions of the penicillin and inhibited at higher ones. The bactericidal action does not take place very rapidly, but in the stronger dilutions it is quite apparent at the end of one hour and nearly complete in from 5 to 10 hours. At slightly

higher dilutions the first effect is one of inhibition for 3 or 4 hours and then gradual sterilization; at still higher levels all these reactions are slower and less complete, and then there is regrowth after 24 or 48 hours. The effect on the bacteria may be, in part, lytic, as definite clearing of cloudy cultures often occurs in the intermediate dilutions, and staphylococcus cultures which have been sterilized by its action were found to contain only a very small amount of granular debris in their sediments. The streptococci killed in this way were still recognizable in the sediment though obviously changed.

Our clinical experience with the crude penicillin is still very limited, but encouraging enough to make us feel that it will find a place in the local therapy of certain infections. Its lack of general or of tissue toxicity, its activity against certain organisms not affected by the sulfonamides, and the fact that it is not inhibited by the body fluids, by pus, nor by para-aminobenzoic acid are points of definite advantage over some of the other chemo- or bio-therapeutic agents.

SUMMARY

1. Methods are described which have been found satisfactory for the production, testing and preservation of crude penicillin.

2. Penicillin is not toxic to animals either by local or parenteral administration.

3. The majority of the organisms tested were Gram-positive cocci; most of these were sensitive to the penicillin but approximately 13 per cent of the staphylococci resisted its action.

4. The action of penicillin is bactericidal in the stronger solutions and inhibitory in the weaker ones.

5. Limited clinical trials in local infections have produced some encouraging results, but have also indicated certain limitations.

6. Penicillin is effective under some conditions which are unfavorable to the action of the sulfonamides and against certain bacteria not sensitive to that group of drugs. It seems likely that even the crude form may prove to be of value in the local therapy of infections.

The author would like to thank the following, for their advice and help: Drs. S. J. Crowe, E. C. White, J. H. Brown, G. O. Gey, R. Austrian and A. T. Ward, Jr.; Miss Clara McKee of the Squibb Institute; Miss M. Kathleen Foley, Mrs. J. K. Polk; and Mr. James Johnson.

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PLATE I

DEMONSTRATION OF BACTERICIDAL ACTION OF CRUDE PENICILLIN (1:20 DILUTION) ON STAPHYLOCOCCUS AUREUS

1. Control plate.
2. After three hours.
3. After six hours.
4. After twenty-four hours (Sterile plate).

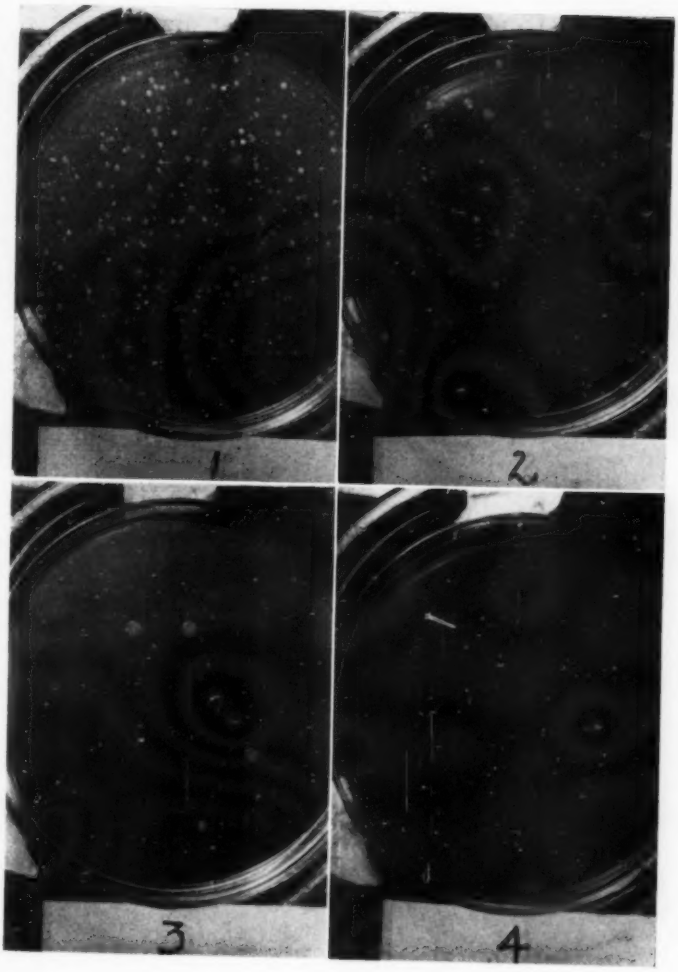

1
2
3
4

PLATE II

BACTERICIDAL ACTION OF CRUDE PENICILLIN ON BETA-HEMOLYTIC STREPTOCOCCUS
(SEE FIGURE 8)

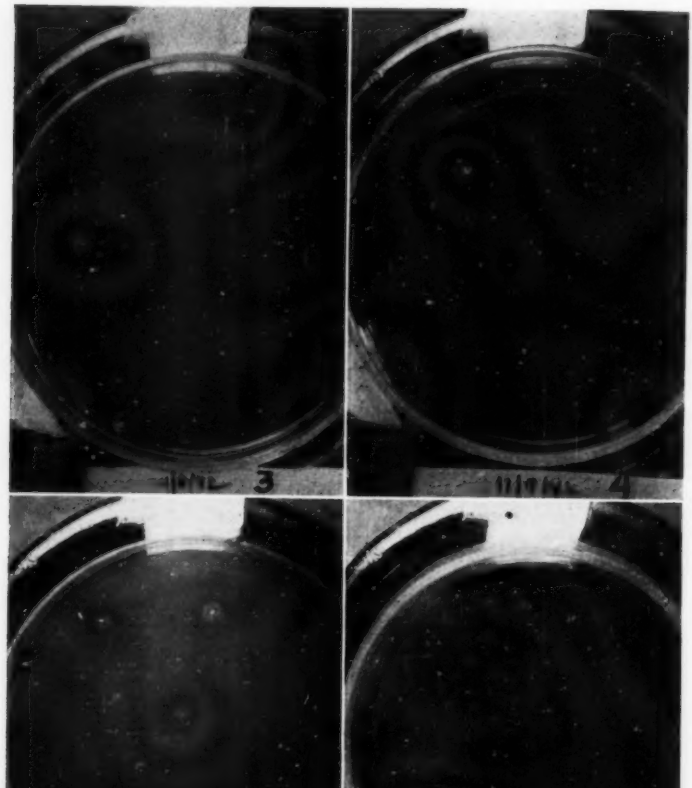
1. Control plate.
2. 1:80 dilution after three hours (Sterile).
3. 1:80 dilution after six hours.
4. 1:80 dilution after twenty-two hours.
5. 1:320 dilution after three hours.
6. 1:320 dilution after six hours.
7. 1:320 dilution after twenty-two hours.

Plate 2 demonstrates the killing action at the lower dilution and the temporary inhibiting effect at the higher dilution.

11/9/32 1

11/9/32 2

11/9/32 3


3
4
6
7

THE FUNGISTATIC AND FUNGICIDAL EFFECT OF SODIUM PROPIONATE ON COMMON PATHOGENS[1]

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Hoffman, Schweitzer and Dalby (1) of the research laboratories of the Ward Baking Company, have demonstrated that many of the fatty acids and their salts are fungistatic for certain non-pathogenic fungi. Many of the large baking companies now incorporate "Mycoban" (trade name of the E. I. DuPont de Nemours Company for sodium or calcium propionate) in bread dough and cake batter to inhibit the growth of mold. It occurred to us that the salts of propionic acid might also inhibit the growth of pathogenic fungi and prove useful in the treatment of both superficial and deep mycotic infections. The fact that the propionates have been incorporated in bread dough and cake batter for human consumption suggested to us that they were not highly toxic. Although there is little literature on the toxicity of the propionates, we have learned that Rowntree (2) injected 20 mg. of sodium propionate daily into rats for nearly a year. He noticed no unfavorable reaction on the animals. This result was confirmed by Dr. William R. Brown of the Philadelphia Institute of Medical Research. Desgrez and Saggio (3) reported that the lethal dose of propionic acid when injected intravenously into a rabbit was 0.45 gm. per kilogram of body weight. This roughly corresponds to a dose of approximately 30 gm. per 150 pounds of body weight. Harshbarger (4) fed the propionates to rats in quantities up to 3 per cent of their diets without noticing a decrease in their weight. This was interpreted to be a measure of the non-toxicity of the propionates.

[1] The work described in this paper was done under a contract, recommended by the Committee of Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

The sodium propionate was generously supplied by the Mycoloid Laboratories, Inc., Little Falls, N. J.

At the very onset it became apparent that the effect of the hydrogen-ion concentration of the sodium propionate solution should be considered. Peck and Rosenfeld (5) working with Trichophyton gypseum, Epidermophyton inguinale, and Candida albicans demonstrated efficient fungistatic action for sodium propionate at a pH of about 5.6. Hoffman, Schweitzer and Dalby (1), working with several species of the Aspergillus and the Penicillium genera demonstrated that the optimum fungistatic properties of propionic acid occurred at a pH range of from 2 to 5.

In a preliminary study we determined that the fungicidal activity of sodium propionate on some pathogenic fungi was exhibited at a pH of about 5.5. Before carrying out laborious laboratory procedures, the clinical effectiveness of a 10 per cent sodium propionate solution adjusted at a pH of 5.5, was tested on patients affected with tinea pedis and tinea cruris. The dramatic way in which the patients responded to treatment stimulated us to test the fungistatic and fungicidal properties of sodium propionate *in vitro* on many of the important pathogenic fungi.

The technique for determining the fungistatic power of sodium propionate was essentially that proposed by Schamberg and Kolmer (6) in 1922. A 25 per cent solution of sodium proprionate adjusted at a pH of 5.5 was added to melted dextrose agar in such amounts as to produce the following concentrations: 0.00125, 0.0125, 0.125, 1.25, 5.0 and 10.0 per cent. Approximately 7 cc. of the medicated agar was placed in clean tubes. The tubes were autoclaved for 15 minutes at 15 pounds of pressure and were slanted. The following day the tubes were seeded with large cuttings (approximately 0.5 cm. square) from freshly isolated fungus cultures and were incubated at room temperature. Two control tubes were made by seeding fungus cuttings on unmedicated dextrose agar. The cultures were observed at 24 hour intervals and the final interpretations were recorded at the end of 21 days. No attempt was made to measure partial inhibition of growth because we felt that the personal ratio of evaluation could only be eliminated by recording the dilution at which complete inhibition had occurred. The results of these experiments are demonstrated in Table 1 and Figures 1 to 5.

The fungus mats that had been used for seeding were removed from those tubes in which complete inhibition had been observed and planted on unmedicated dextrose agar slants. The cultures were incubated at room temperature for 21 days and then examined for growth. We were, thereby, able to ascertain for each fungus tested, the concentration of sodium propionate necessary for fungicidal activity. The results are demonstrated in Table 2.

Control
Sod. Propionate 1.25%
Control
Sod. Propionate 1.25%
Control
Sod. Propionat. 1.25%
Aspergillus flavus
Microsporum audouini

FIG. 1

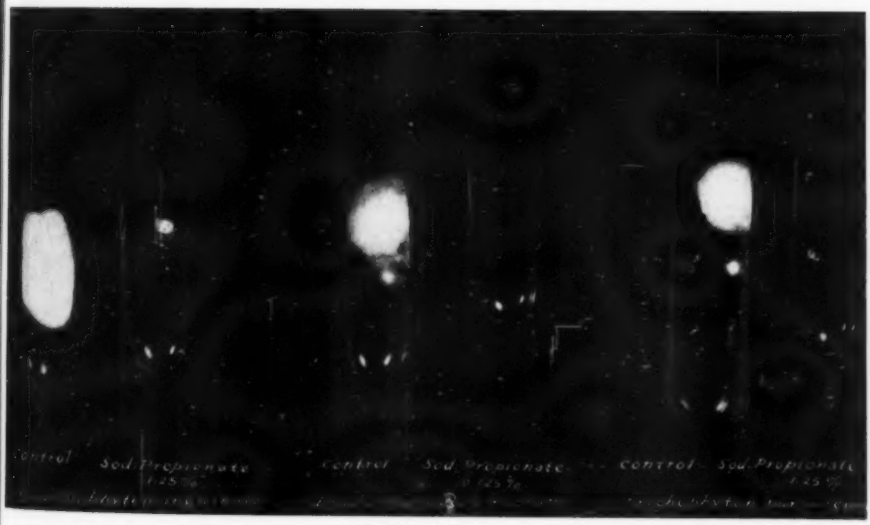
Control
Sod. Propionate 1.25%
Control
Sod. Propionate 1.25%
Control
Sod. Propionate 1.25%

FIG. 2

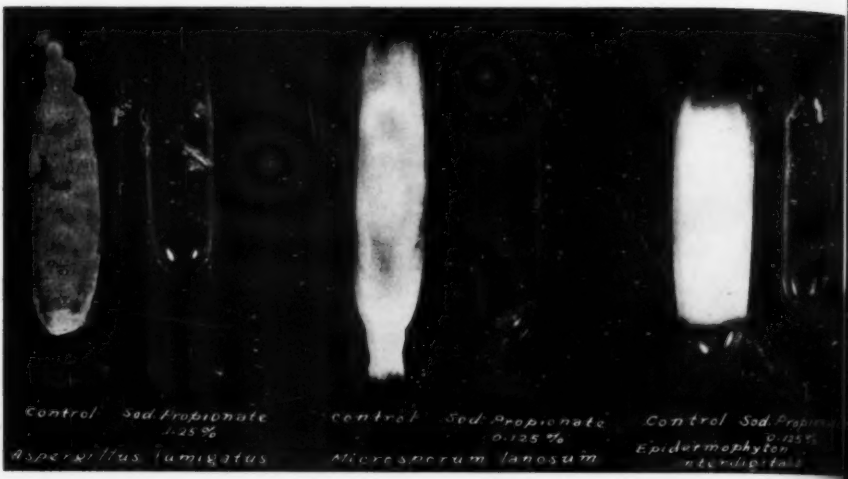
Control
Sod. Propionate 1.25%
Aspergillus fumigatus
control
Sod. Propionate 0.125%
Microsporum lanosum
Control
Sod. Propionate 0.125%
Epidermophyton interdigitale

FIG. 3

Control
Sod. Propionate 0.125%
control
Sod. Propionate 1.25%
control
Sod. Propionate 0.125%

FIG. 4

It must be emphasized, however, that the fungus mats had been in constant contact with the medicated dextrose agar for 21 days before being removed and it seemed essential then to determine the fungicidal activity of sodium propionate for shorter exposure intervals.

Various techniques for the testing of fungicidal power as suggested by Schamberg and Kolmer (6), Emmons (7), Sharlit and Muskatblit (8), Smyth and Smyth (9) and Burlingame and Reddish (10), were reviewed. We chose the technique suggested by Burlingame and Reddish (10) because it offered a fairly simple but severe test which more closely approached conditions met with in clinical practice. We are fully aware, however, that even in this test the penetrating power of the

FIG. 5

chemical in dextrose agar cannot be compared with its penetrating power in the keratin of the skin. This naturally leaves much to be desired in the performing of *in vitro* tests.

Twenty cc. of melted dextrose agar were placed in 9 cm. petri dishes and each dextrose agar plate was inoculated with a freshly isolated culture by streaking the entire surface of the plate with a dry, sterile cotton swab. The plates were incubated at room temperature for 5 days. The agar cultures were then cut into 1 cm. squares and about 15 cc. of the sodium propionate solution to be tested was poured over each plate so that the cultures were flooded. After periods of 5, 15, 30 and 60 minutes and 24 hours, the squares of culture and agar were removed

TABLE 1

Demonstrating the Fungistatic Activity of Sodium Propionate, Adjusted to pH 5.5, on Common Pathogenic Fungi

TYPES OF FUNGI	SODIUM PROPIONATE (PERCENTAGE AT WHICH GROWTH WAS COMPLETELY INHIBITED)
Trichophyton gypseum	0.125
Trichophyton purpureum	1.25
Trichophyton violaceum	0.125
Trichophyton schoenleini	0.125
Epidermophyton rubrum	0.125
Epidermophyton inguinale	1.25
Epidermophyton interdigitale	0.125
Microsporum lanosum	0.125
Microsporum audouini	1.25
Candida albicans	1.25
Torula histolytica	1.25
Blastomyces dermatitidis	0.0125
Coccidioides immitis	1.25
Aspergillus fumigatus	1.25
Aspergillus flavus	1.25
Actinomyces bovis	2.00

Since the time that we submitted this article for publication we have found that a 0.125 per cent solution of sodium propionate, adjusted to pH 5.5, also completely inhibits the growth of Sporotrichum schencki, Phialophora verrucosa and Histoplasma capsulatum.

TABLE 2

Demonstrating the Fungicidal Activity of Sodium Propionate, Adjusted to pH 5.5, on Common Pathogenic Fungi (Fungus Cultings Remained on Medicated Dextrose Agar for Three Weeks)

TYPES OF FUNGI	SODIUM PROPIONATE (PERCENTAGE AT WHICH GROWTH WAS KILLED)
Trichophyton gypseum	1.25
Trichophyton purpureum	1.25
Trichophyton violaceum	1.25
Trichophyton schoenleini	0.125
Epidermophyton rubrum	1.25
Epidermophyton inguinale	1.25
Epidermophyton interdigitale	1.25
Microsporum lanosum	0.125
Microsporum audouini	1.25
Candida albicans	5.0
Torula histolytica	1.25
Blastomyces dermatitidis	1.25
Coccidioides immitis	10.0
Aspergillus fumigatus	1.25
Aspergillus flavus	1.25

from the plates and placed in 10 cc. of sterile dextrose broth. The excess fungicide was washed from the matted cultures by shaking the broth tubes lightly for a period of 5 minutes. At the end of this time the blocks of culture and agar were removed from the broth and placed with the culture side down on the surface of sterile dextrose agar plates. The plates were incubated at 30°C. for 3 weeks and were then observed for growth.

The purpose of these experiments was to determine at what pH and at what concentration sodium propionate was most fungicidal for each fungus tested. It was soon observed that the optimum pH was 5.5, 6, or 6.5 and that the optimum concentration was a 10 to 20 per cent solution. The results of these experiments are summarized in Table 3.

DISCUSSION

It is impossible to predict or anticipate the clinical effectiveness of fungistatic or fungicidal agents from the results of *in vitro* experiments. However, the knowledge of such experiments assists the investigator in selecting the concentration of the drug and the hydrogen-ion level that may be effective for clinical experimentation.

The fungi included in the genera of Trichophyton, Epidermophyton, Microsporum and Candida, commonly referred to as "the dermatophytes", infect the superficial layers of the skin and reside for the most part in the stratum corneum. The inflammatory reaction in the surrounding tissues is actually an allergic reaction to the infecting fungi or their products. Some immunity on the part of the host is established as a result of this superficial infection. A chemical used in the treatment of such an infection must possess the ability to penetrate the stratum corneum and come in contact with the hyphae that are imbedded there.

It is a matter of common knowledge that the superficial mycotic infections of the skin are likely to recur. This is particularly so for tinea pedis. There are two possible explanations for this. One is that reinfection takes place by contagion and the other is that the fungus lies dormant in the tissues, awaiting a period of breakdown in the host's resistance which will permit its propagation. Therefore, a chemical that is to be employed to cure and prevent infection must be used frequently over a long period of time. Sodium propionate promises to be non-toxic for the host and, as a subsequent report will verify, can be used daily without irritation for many months. In the treatment of the deep mycotic infections it is paramount that

TABLE 3

Demonstrating the Fungicidal Activity of Sodium Propionate, at Various pH levels and in different Concentrations for Various Exposure Intervals, on Common Pathogenic Fungi

TYPES OF FUNGI	EXPOSURE INTERVAL	pH 5.5		6.0		6.5		7.0		7.5	
		Concentration									
		10%	20%	10%	20%	10%	20%	10%	20%	10%	20%
Trichophyton gypseum	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+	+
Trichophyton purpureum	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	0	+	+	+	+	+	+	+	+
	60 min.	+	0	+	+	+	+	+	+	+	+
	24 hrs.	0	0	0	0	+	0	+	+	+	+
Trichophyton violaceum	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+	+
Trichophyton schoenleini	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	0	+	+	+	+	+	+	+	+
	24 hrs.	+	0	+	+	+	+	+	+	+	+
Microsporum audouini	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	0	+	0	+	+	+	+	+	+
Candida albicans	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+	+
Torula histolytica	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+	+
Blastomyces dermatitidis	5 min.	+	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+	+
Coccidioides	5 min.	+	+	+	+	+	+	+	+	+	+

Epidermophyton rubrum	5 min.	+	+	+	+	+	+	+	+	+	Coccidioides immitis	5 min.	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+		15 min.	+	+	+	+	+	+	+	+	+
	30 min.	+	0	+	+	+	+	+	+	+		30 min.	+	+	+	+	+	+	+	+	+
	60 min.	+	0	+	+	+	+	+	+	+		60 min.	+	+	+	+	+	+	+	+	+
	24 hrs.	+	0	+	0	+	0	+	+	+		24 hrs.	+	+	+	+	+	+	+	+	+
Epidermophyton inguinale	5 min.	+	+	+	+	+	+	+	+	+	Aspergillus fumigatus	5 min.	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+		15 min.	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+		30 min.	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+		60 min.	+	+	+	+	+	+	+	+	+
	24 hrs.	+	+	+	+	+	+	+	+	+		24 hrs.	+	+	+	+	+	+	+	+	+
Epidermophyton interdigitale	5 min.	+	+	+	+	+	+	+	+	+	Aspergillus flavus	5 min.	+	+	+	+	+	+	+	+	+
	15 min.	+	+	+	+	+	+	+	+	+		15 min.	+	+	+	+	+	+	+	+	+
	30 min.	+	+	+	+	+	+	+	+	+		30 min.	+	+	+	+	+	+	+	+	+
	60 min.	+	+	+	+	+	+	+	+	+		60 min.	+	+	+	+	+	+	+	+	+
	24 hrs.	+	0	+	+	+	+	+	+	+		24 hrs.	+	+	+	+	+	+	+	+	+
Microsporum lanosum	5 min.	+	+	+	+	+	+	+	+	+											
	15 min.	+	+	+	+	+	+	+	+	+											
	30 min.	+	+	+	+	+	+	+	+	+											
	60 min.	+	+	+	+	+	+	+	+	+											
	24 hrs.	0	0	+	+	+	+	+	+	+											

+ signifies growth of fungus (no fungicidal activity). 0 signifies absence of growth of fungus (fungicidal activity).

the therapeutic agent be non-toxic for the host because the chemical, to be effective, must be given parenterally or orally. We have reason to believe at this time that sodium propionate can be administered in such ways with impunity.

From the results of the experiments summarized in Table 1, a 0.125 per cent solution of sodium propionate inhibits completely the growth of Trichophyton gypseum, Trichophyton violaceum, Trichophyton schoenleini, Epidermophyton rubrum, Epidermophyton interdigitale, Microsporum lanosum, and in a dilution of 1.25 per cent it completely inhibits Trichophyton purpureum, Epidermophyton inguinale, and Microsporum audouini. These organisms represent the common dermatophytes. Candida albicans, Torula histolytica, Coccidioides immitis, Aspergillus fumigatus and Aspergillus flavus are completely inhibited by a 1.25 per cent solution of sodium propionate and Blastomyces dermatitidis is completely inhibited by 0.0125 per cent solution of sodium propionate. A 2 per cent solution of sodium propionate prepared in thioglycollate media completely inhibited the growth of Actinomyces bovis.

The results taken from Table 2 indicate that the same or slightly stronger solutions of sodium propionate that had been used to inhibit completely fungus growth also killed the fungi sometime during the 3 week period of incubation.

The results tabulated in Table 3 demonstrate the fungicidal activity of sodium propionate for short intervals of exposure and it is necessary to stress that under such conditions sodium propionate is fungicidal only in solutions of 10 and 20 per cent and then only when the pH is adjusted to 5.5 or 6.0. Even in such strong solutions and at such hydrogen-ion concentration sodium propionate was not fungicidal for Trichophyton gypseum, Trichophyton violaceum, Epidermophyton inguinale, Coccidioides immitis, Candida albicans, Blastomyces dermatitidis, Torula histolytica, Aspergillus fumigatus and Aspergillus flavus.

CONCLUSIONS

The fungistatic activity of sodium propionate for various strains of common pathogenic fungi has been determined according to the method suggested by Schamberg and Kolmer. Adjusted at a pH of

5.5 sodium propionate was found to be fungistatic in a 1.25 per cent solution for Trichophyton purpureum, Epidermophyton inguinale, Microsporum audouini, Candida albicans, Torula histolytica, Coccidioides immitis, Aspergillus fumigatus and Aspergillus flavus and in a 0.125 per cent solution for Trichophyton gypseum, Trichophyton violaceum, Epidermophyton rubrum, Epidermophyton interdigitale, Trichophyton schoenleini, and Microsporum lanosum; and in a 0.0125 per cent solution for Blastomyces dermatitides. It was fungistatic for Actinomyces bovis in a solution of 2 per cent.

After 3 weeks contact with the fungus mats, sodium propionate adjusted to a pH of 5.5 was fungicidal in a 0.125 per cent solution for Trichophyton schoenleini and Microsporum lanosum; in a 1.25 per cent solution for Trichophyton gypseum, Trichophyton purpureum, Trichophyton violaceum, Epidermophyton rubrum, Epidermophyton inguinale, Epidermophyton interdigitale, Microsporum audouini, Torula histolytica, Blastomyces dermatitidis, Aspergillus fumigatus and Aspergillus flavus; in a 5 per cent solution for Candida albicans, and in a 10 per cent solution for Coccidioides immitis.

The technique of Burlingame and Reddish was also employed to determine the fungicidal activity of sodium propionate. By this method optimum fungicidal activity existed only in solutions of 10 and 20 per cent adjusted at pH 5.5, 6.0 and 6.5 for Trichophyton purpureum, Trichophyton schoenleini, Epidermophyton rubrum, Epidermophyton interdigitale, Microsporum lanosum and Microsporum audouini. No fungicidal activity was demonstrated for Trichophyton gypseum, Trichophyton violaceum, Epidermophyton inguinale, Candida albicans, Blastomyces dermatitidis, Coccidioides immitis, Torula histolytica, Aspergillus fumigatus and Aspergillus flavus.

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GUILLAIN-BARRÉ SYNDROME (ACUTE INFECTIVE POLYNEURITIS) WITH INCREASED INTRACRANIAL PRESSURE AND PAPILLEDEMA

Report of Two Cases

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The purpose of this paper is to call attention to the occasional occurrence of increased intracranial pressure in the course of infective polyneuritis, or polyradiculoneuritis as some authorities prefer to term it. This condition is really a very common one but there is so much confusion about the criteria necessary for the diagnosis and so many different names are employed to designate it that its incidence is usually underestimated. Among the terms applied to this condition are Landry's paralysis, acute febrile polyneuritis, acute infective polyneuritis, infective neuronitis, motoneuronitis, polyneuritis with facial diplegia and Guillain-Barré syndrome. The diagnosis depends upon the presence of certain characteristic features. Various writers have emphasized one or more of these characteristics. For example, Landry (1) described the rapid and extensive paralysis of the extremities and trunk and pointed out that the patient's mind remained clear to the end even in fatal cases. The tendency of the weakness to involve the proximal muscle groups more severely than the distal muscle groups in certain cases was stressed by Foster Kennedy (2). The development of bilateral facial paralysis was regarded as typical by Patrick (3), Viets (4) and others. The history of a brief febrile illness shortly before the onset of the paralysis was noted by Osler (5) and Holmes (6). Guillain, Barré and Strohl (7) showed that there is a great increase of the protein content of the spinal fluid in some cases (8) and the term Guillain-Barré syndrome has been applied to such cases since their paper was published in 1916. We wish to add one more element to this picture and to point out that increased intracranial pressure may occur in cases otherwise typical of the

Guillain-Barré syndrome. It is scarcely necessary to add that the cause of this condition, if indeed there is one cause and not many causes, is quite unknown. It is generally assumed that an infection is to blame.

A review of the literature dealing with infective polyneuritis reveals few references to papilledema. Gilpin *et al.* (9) state that three patients in their series showed papilledema and Mirus (10) mentions bilateral papilledema of three diopters in one of his cases. Occasional notes of "optic neuritis" are found but the descriptions are not sufficient to enable one to determine whether there was papilledema or not.

Case History 1 (U-280467).[1] *While woman of 26 years developed slowly progressive paralysis of her extremities with facial paralysis. Symptoms began in the 4th month of pregnancy, and reached their maximum shortly after delivery. During the 7th month of the illness the patient complained of headache and was found to have bilateral papilledema. Spinal fluid pressure 600 mm. H_2O. Total protein 470 mgms. per 100 cc.*

H. B., a young married woman of 26 years, became pregnant in January 1942. Aside from some vomiting in the early weeks she was quite well until about the beginning of the fourth month when she noticed numbness and tingling in the hands and feet. These symptoms slowly grew worse and as term approached she was scarcely able to walk. After delivery she was helpless and confined to bed. Her arms were weak, especially in the distal joints and she was unable to move her legs at the knee or ankle joints. Facial paralysis more evident on the left than the right appeared a month after the onset but passed off in a few weeks.

A few weeks after delivery, improvement began and continued at a slow pace. Some two months after delivery the patient began to complain of headache and of blurring of vision. Her local physician discovered papilledema and she was sent to Dr. Dandy.

Examination on January 8, 1943 revealed a well developed and well nourished young woman who was mentally clear and intelligent. There was paralysis of all movements at the ankle and toe joints. At the knee joints, both flexion and extension were very weak but not lost and at the hip all movements were slightly weak. The arms were strong in all muscle groups except for the intrinsic muscles of the hands which were slightly weak. There was scarcely any muscular wasting. The tendon reflexes were all absent as were the plantar and abdominal reflexes. The calves were slightly tender and a little hypesthesia was found over the feet. Sense of position of the toes was reduced.

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[1] We wish to thank Dr. Dandy for permission to report this case.

The optic nerve heads showed papilledema of 3 diopters with marked engorgement of the veins and filling of the cups. There were no hemorrhages or exudates. Vision was normal and the fields full.

Spinal fluid pressure was 600 mm water. It contained 470 mgm. protein per 100 cc. There were no cells. The Wassermann reaction in both the blood and spinal fluid was negative. A ventriculogram was performed. This showed a symmetrical ventricular system of average size.

In view of the papilledema and the greatly increased spinal fluid pressure, decompression was considered necessary to save the patient's vision. This was performed on January 15. The brain was normal in appearance, but herniated strongly when the bone was removed.

Subsequently the papilledema receded and the headaches ceased. The patient reports continued but slow improvement in strength.

Case History 2 (U-243516). *White boy of 16 yrs. developed paralysis of all 4 extremities in the course of 7 months. Improvement then began. Some 8 months after the onset there was bilateral papilledema and a spinal fluid pressure of 400 mm. water. Spinal fluid contained 200 mgs. protein per 100 cc.*

T. G., a boy of 16 years, noticed gradually increasing weakness of the arms and legs in November 1940. There was no history of a preceding illness. During the next few months the weakness slowly increased and in June, 1941 he was completely paralysed in all four extremities. His diaphragm acted strongly but the intercostal muscles and the muscles of the spine were paralysed. There was only an occasional pain in the calves and a little tenderness of the affected muscles. Throughout this illness the patient complained of frequent and severe headaches and had bouts of vomiting from time to time.

In July he began to improve and soon regained some power of contraction in all groups of muscles. The improvement continued but progress was very slow.

In October 1941 he was brought to the Johns Hopkins Hospital. Examination revealed generalized weakness of all four extremities. The weakness was diffuse and symmetrical, involving all muscle groups about equally. There was no actual paralysis but the weakness was so severe he could not lift his extremities from the bed. There was a moderate degree of atrophy of the muscles. The tendon reflexes were absent as were the abdominal and plantar responses. A little hypesthesia was present over the feet and ankles and the calves were tender to pressure. Sense of passive movement and vibratory sense were lost in the legs. There was no loss of control of the bladder or bowels. His mental condition was not altered.

The optic nerve heads showed papilledema of moderate degree with partial filling of the cups and blurring of the disc margins. There were no hemorrhages or exudates. Vision was normal and the fields of normal extent. The cranial nerves were otherwise normal.

The spinal fluid was clear and contained only 2 cells per cmm. The pressure

was 400 mm water. The total protein content was 200 mg. per 100 cc. The Wassermann test of the blood and spinal fluid was negative. The Queckenstedt test showed no evidence of obstruction of the spinal canal or lateral sinuses. Roentgenograms of the chest revealed nothing abnormal. The long bones showed merely atrophy. Blood sugar was 76 mg. per 100 cc. Non-protein nitrogen in the blood was 32 mg. per 100 cc. Tests for agglutinins against common bacteria were negative. The basal metabolic rate was minus 5. Red blood cells numbered 5,000,000, white blood cells 9850. An electroencephalogram was made but the record was normal in all respects.

The patient was discharged after remaining in the hospital for five days. He has written since to say that the headaches disappeared some months after he left the hospital and that there has been slow improvement in strength. A year after he was examined, he was still unable to walk.

SUMMARY AND CONCLUSIONS

Two cases of Guillain-Barré Syndrome are reported. The neurological findings were typical and the spinal fluid contained a great excess of protein. In each case there was abundant evidence of increased intracranial pressure. The pressure measured 400 mm. of water in one and 600 mm. water in the other. Papilledema was present in each case. A ventriculogram was performed in one case and showed ventricles of normal size and shape. An electroencephalogram was made in one case with negative results. In neither patient was there any mental disorder present. No symptoms of increased intracranial pressure were noted except for headache and vomiting.

We have found in the medical literature mention of four cases in which papilledema occurred in association with the Guillain-Barré syndrome.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Biochemistry and Morphogenesis. By JOSEPH NEEDHAM. 787 pp. Illus. $12.50. Cambridge: at the University Press; New York: The Macmillan Company, 1942.

This recent work of the British biochemist gives a comprehensive picture of the present knowledge of the biochemical facts and theories related to the morphogenesis of organisms. In contrast to the author's earlier work, "Chemical Embryology", in which interest was focussed on the chemical changes during embryonic development, the central subject of the present contribution is the problem of the biochemical causation of morphogenesis. This new approach was ushered in by the demonstration of embryonic inductions with non living cell material by Spemann in 1931, the year of the publication of "Chemical Embryology".

The book is divided into three parts. Part I deals with the "morphogenetic substratum"; i.e. with the chemical raw materials and the nutrition of the embryo. The metabolic relation of the egg to its environment is discussed in detail. Of particular interest is the concept of the uricotelic nitrogen metabolism of birds and reptiles as an evolutionary corollary to the non aquatic environment of their eggs.

Part II deals with the "morphogenetic stimuli". Here it is attempted to endow the morphological description of the organiser phenomena (evocation and individuation) with a biochemical meaning. The chemical nature of the primary organiser and its relation to known substances of steroid nature (sex hormones, carcinogens) as well as the nature of the inactive evocator complex are discussed. The relationship between the presence of free evocator, of competence, and of the "individuation field" forms the basis for an interesting discussion on the morphogenesis of twinning, of teratomata, and of malignant growth. Second and third grade organisers, and the relation of genes to organiser phenomena are reviewed.

Part III deals with the "morphogenetic mechanisms". Here the integration and dissociability of the fundamental processes in morphogenesis are discussed. Chemical heteroauxesis is illustrated by many examples. A thorough account of the respiration and of the metabolism of carbohydrates, proteins, nucleins, lipins and pigments during development is given. It may be questioned whether the growth-promoting factor (or factors) should not be discussed in the section on "morphogenetic stimuli".

Phenomena at the molecular level on the one hand and those at the microscopic or gross morphological level on the other hand form the main subjects of this book. However, the author is keenly aware that the appreciation of phenomena on an intermediate level of organization is indispensable for a better understanding of the organizational problems of biomorphogenesis. A special section on "polar-

ity" is dedicated to this intermediate level with a discussion of the paracrystalline state, of fibre molecules, and of the anisometropic protein molecules in eggs and embryos.

The book will stimulate the worker in any field of experimental biology concerned with problems of growth and development, as well as the geneticist, endocrinologist and pathologist who is not experimentally oriented. The huge amount of assembled data, to which the author and his associates have contributed substantially by their experimental work, and the carefully compiled bibliography and indices will make this book indispensable as a reference work. The book is well illustrated. The glossary of special terms renders the text readily understandable to the reader who is not trained in the terminology of experimental embryology. The occasionally speculative character of the presentation may be appreciated as the groping of a very young branch of scientific development, of that recent period which is marked by the growing understanding of a very close relation between biochemistry and morphology.

The fundamental nature of the approach and of the author's concept of morphogenetic organization makes this book valuable beyond its importance as a monograph on a special field of biological science; abstracting from the problem of organization at the level of the individual organism, the author concludes his work fittingly and confidently: "Human society itself in that slow but persistent and inevitable rise in level of organization which biological and social evolution represents, mirrors . . . the ontogenetic rise of the individual organism in organizational level. Let us hope that human society will before long attain a level of organization such as to encourage and facilitate the great enterprise of embryology. The study of the beginnings of life has something in it antithetical to death, the harmonious relations of morphogenesis something inimical to human disunity and hatred, the co-operation of embryonic processes something utterly alien to human oppression and war".

W. B.

Principles and Practice of War Surgery. By J. TRUETA. 441 pp. Illus. $6.50. The C. V. Mosby Company, St. Louis, 1943.

This book of 425 pages includes 144 illustrations and a moderately comprehensive bibliography. Due acknowledgement is made in the introduction to the work of Winnett Orr and of many others who have contributed to the field. The best part of the book is that which deals with the five points of importance in the Trueta technique; namely, (1) prompt surgical treatment, (2) cleansing of the wound, (3) excision of the wound, (4) provision of drainage, and (5) immobilization in a plaster-of-Paris cast. The reasons for each step are given very clearly. The advantages of the method, according to Trueta, include the protection of the wound against secondary infections, the pressure on the surface of the wound, the prevention of damage to granulation tissue, the absorption by the plaster of excessive discharge which might damage the newly formed epithelial cells, the avoidance of edema, the maintenance of a good circulation, and above all the absolute and con-

tinuous rest of the affected limb. A combination of all these factors forms the fundamental basis for the success of closed plaster casts in the promotion of wound healing.

The book also contains a consideration of other subjects such as shock, transfusions, tetanus, gas gangrene, chemotherapy, anesthesia, skin grafting and the treatment of burns. Trueta's treatment of these subjects is not on a par with those chapters dealing with the general care of wounds. The chapter on shock is particularly poor.

All in all, the book is worth while and is recommended highly, particularly to those who will treat injuries under conditions imposed in time of war.

A. B.

Gynecology, with a section on Female Urology. By LAWRENCE R. WHARTON. 1006 pp. Illus. $10.00. W. B. Saunders Company, Philadelphia & London, 1943.

This single volume text and reference book is as comprehensive and complete as a single volume work covering the two subjects indicated by the title can be. The first 154 pages are devoted to embryology, anatomy and physiology. Approximately the next 60 pages are logically devoted to gynecological conditions upon which physiology has a direct bearing: functional bleeding, dysmenorrhea and the menopause. A short section follows on methods of gynecological examination. After this there are sections covering over 500 pages devoted to clinical gynecology. The remainder of the 1000 page volume covers the subject of female urology from both a diagnostic and the therapeutic standpoint.

The book is well illustrated. Many new drawings are reproduced but the majority have been taken from recent literature and earlier published works, illustrated in many instances by Max Brödel. The new drawings by Schlossberg are excellent examples of clean cut medical illustrating.

The book deserves a wide distribution for it is concise enough for the busy student or practitioner and comprehensive enough for a reference work for the young aspiring gynecologist.

R. W. TEL.

Methods for Diagnostic Bacteriology. By ISABELLE G. SCHAUB and M. KATHLEEN FOLEY. 2nd ed. 430 pp. $3.50. The C. V. Mosby Company, St. Louis. 1943.

This book has been found in the past to be of immense value, and constitutes "a bible" which should be in every diagnostic bacteriology laboratory.

The second edition contains much that is new. Most important in this respect is an entire chapter on "Identification of Pathogenic Bacteria by Colony Characteristics". To the reviewer's knowledge this is the only book that contains all the information on colony characteristics in readily accessible and concentrated form, Among other new items are included material on Neisseria gonorrhoeae, isolation of anaerobes, yeast and fungus cultures and uterine cultures. All the advan-

tageous features of the first edition remain intact and the index has been improved. The authors are to be commended on the manner in which they have brought their book up to date.

G. H.

Health Education of the Public. By W. W. BAUER and THOMAS G. HULL. 2nd ed. 315 pp. Illus. $2.75. W. B. Saunders Company, Philadelphia and London, 1942.

Dr. Bauer and Dr. Hull have prepared a revision of their manual "Health Education for the Public", with additional information and certain new features and ideas. This manual serves as a guide to the handling of health education problems, and offers the solutions of two men who are constantly encountering situations in their contact with the public through the radio and the press. The various methods or techniques which may be employed are given in sufficient detail to indicate the pitfalls which may entrap the inexperienced or unwary worker in this field. The authors emphasize the need to weld these techniques into a well balanced program adapted to local circumstances and local needs. Certain crude indices are suggested for use in the measurement of results. This is a step in the proper direction, but it is unlikely that the yardsticks chosen would give valid and practical results.

J. J. P.

Miotonias. By ALFREDO LANARI. 277 pp. Illus. "El Ateneo," Buenos Aires. [n.d.].

The author, an Associate of the Hospital Nacional de Clinicas, of Buenos Aires, has published the results of seven years' work on Myotonia.

After discussing the history and etiology the writer gives a careful description of the different symptoms. There is an interesting account of his own experiences and investigations. Under the titles of Pathological Anatomy and Pathogenesis of the Myotonic Syndrome the author reviews the most recent investigations and conclusions. The book with its extensive bibliography gives one of the most complete accounts of the work done in the recent years on Myotonias. It should be said, too, that the book is well written. Finally the editor deserves congratulations on the clean and beautiful appearance of the volume.

L. O.